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Effects of Passive Smoking on Spirometric Values and Plasma Level of Inflammatory Markers in Healthy School Children in Khartoum

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Dedication

To all members of my family

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I would like to express my gratitude to all those who gave me the possibility to complete this thesis. I want to thank the Department of Physiology of Khartoum University for giving me permission to commence this thesis in the first instance, to do the necessary research work and to use departmental equipments.

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Abbreviations

WHO	World Health Organization
FVC	Forced Vital Capacity
FEV1	Forced Expiratory Volume in the First Second
FEF25%	The Forced Expiratory Flow 25%
FEF50%	The Forced Expiratory Flow 50%
FEF25 -75%	The Forced Expiratory Flow 25-75%
PEF	The Peak Expiratory Flow Rate
BTS	British Thoracic Society
ATS	American Thoracic Society
USA	United states of America
IL	Interleukin
CRP	C-Reactive Protein
TNFα	Tumor Necrosis Factor α
IFNγ	Interferone γ
IgE	Immunoglobulin E
COPD	Chronic Obstructive Pulmonary Disease
BMI	Body mass index

Abstract

Background: Exposure of children to environmental tobacco smoke at home has been linked to many complications including respiratory symptoms, airway inflammation and impaired lung ventilatory functions; however, factors like housing conditions and genetic predisposition may influence the development of these complications.

Objectives: To determine the effects of passive smoking on spirometric values and plasma level of inflammatory markers in healthy male school-children.

Methods: This is an observational case control study conducted in year 2009 among 135 male school-pupils (69 cases and 66 controls), aged 9-14 (mean 11.6 ± 1.3) years, selected randomly from three governmental primary schools for boys in Khartoum. Information about smoking history, its impact on child's health and the attitude of parents towards passive smoking was collected from each pupil and his parents using a questionnaire. Spirometric measurements were performed using Clement Clarke All-flow Spirometer. Plasma CRP, IL4 and TNFα were measured using ELISA kits.

Results: Fathers were responsible for 67.5% of second-hand smoke at home; mothers were responsible for 2.5% whereas relatives like brothers and uncles were responsible for 30%. The group of cases showed a significant rise in plasma TNFα and IL4 and a significant reduction in FVC and FEV_1 by about (7-8%). The rise in plasma TNFα and IL4 was higher in those with high degree of tobacco smoke exposure than those with low exposure, indicating a dose dependent effect. No significant difference was

found in frequencies of hospital admissions, surgical operations and respiratory tract infections during the last year; however, snoring during sleep was significantly higher among cases (8.2%) than controls (2.2%). The majority of fathers in the study group did not smoke or allow smoking near their wives during pregnancy (90%).

Conclusion & recommendation: Passive smoking has a significant negative impact on lung function of healthy male school-pupils in Khartoum and a significant dose dependent effect on plasma TNFα and IL4.Educational programs are highly recommended to increase awareness of parents about the negative impact of passive smoking on child's health.

ملخص الاطروحة

الخلفية : قد تم ربط تعرض الأطفال لدخان التبغ البيئي في المنزل بالكثير من المضاعفات التي تتضمن أعراض تنفسية ، التهاب الشعب الهوائية وضعف وظائف تهوية الرئة ولكن عوامل مثل ظروف السكن والاستعداد الوراثي قد تؤثر على تطور هذه المضاعفات.

الأهداف : لتحديد تأثير التدخين السلبي على قيم معايير مقياس التنفس ومستوي مؤشرات الالتهاب في بلازما أطفال المدارس الأصحاء الذكور.

الأساليب : هذه دراسة مراقبة ، حالة ضابط ، أجريت في عام 2009 في 135 تلميذ مدرسة (69 حالة و 66 ضابط) ، تتراوح أعمارهم بين 9-14 (المتوسط 11.6 ± 1.3) سنوات ، تم اختيارهم عشوائيا من ثلاث مدارس حكومية ابتدائية للبنين في الخرطوم. المعلومات عن تاريخ التدخين وتأثيره على صحة الطفل وموقف الآباء تجاه التدخين السلبي تم جمعها من كل من التلميذ وعائلته باستخدام استبيان. قياسات قيم معايير التنفس أجريت باستخدام مقياس تنفس من كليمنت كلارك. تم قياس IL4 و TNF و CRP في البلازما باستخدام مجموعات الاليزا.

النتائج : الآباء كانوا مسؤولين عن 67 ٪ من دخان التبغ غير المباشر في المنزل ؛ الأمهات كن مسؤولات عن حوالي 2 ٪ في حين أن الأقارب مثل الاخوة والأعمام كانوا مسؤولين عن حوالي 30 ٪. مجموعة الحالات كشفت عن ارتفاع ملحوظ في TNFα و IL4 و انخفاض ملحوظ في (FVC، FEV1) بحوالي 7-8%. الارتفاع في TNFα و IL4 البلازما كان أعلي في الذين تعرضوا لدرجة عالية من دخان التبغ من الذين تعرضوا لدرجة منخفضة ، مما يشير الي تأثير يعتمد علي الجرعة. لا يوجد فرق كبير في تواتر حالات دخول المستشفيات والعمليات الجراحية والتهابات الجهاز التنفسي خلال العام الماضي ، إلا أن الشخير أثناء النوم كان مرتفعا بشكل كبير بين الحالات (8.2 ٪) مقارنة بالضوابط (2.2 ٪). غالبية الآباء في كل مجموعة الدراسة لا يسمحون بالتدخين قرب زوجاتهم خلال فترة الحمل (90 ٪).

الاستنتاج والتوصية : التدخين السلبي له تأثير مقدر على مستوى مؤشرات الالتهاب في البلازما وله ارتباط ضعيف مع قيم معايير مقياس التنفس في تلاميذ المدارس الذكور في الخرطوم. البرامج التعليمية موصي بها بقوة لزيادة الوعي حول التأثير الضارللتدخين السلبي على صحة الطفل.

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CHAPTER ONE

1. INTRODUCTION & LITERATURE REVIEW

1.1 Cigarette smoking

1.1.1. History

Smoking was probably first practiced by the indigenous people of the Western Hemisphere. Originally used in religious and social rituals and in some instances for medical purposes.(1,2) By the time Europeans arrived on the American continent in the fifteenth century, smoking of tobacco was popular among the natives. Christopher Columbus and his sailors were given tobacco, among other gifts, by the American Indians in 1492. Then tobacco leaves and seeds were brought back with them to Europe for the first time.(1) By the end of the sixteenth century, smoking of tobacco had became a widespread practice.(1,2) However, many rules prohibited its use and penalized offenders. Near the end of the nineteenth century, mass production of cigarettes had begun. Following this, smoking of cigarettes gradually became prevalent as the use of cigars and pipes declined.(1-3)

During the early stages of the smoking epidemic, and before the mass production of cigarettes, women were not regarded as smokers in public although some of them did smoke in private.(4) In the 1920s, the tobacco companies began to attract women as smokers through media and cigarette advertising. This contributed to a culture in which smoking was normalized and had a positive image. Since that time the behavior of women was changed and female smoking started to rise.(4)

In the Sudan, nobody knows exactly where and how tobacco was introduced, but the archeological excavations in the Northern part of the country discovered the use of tobacco pipes dating back to Christian era (6^{th}-14^{th} century A.D.).(5) The earliest recorded information is very recent;

early nineteenth century by the Turks. Scattered small areas were grown with varieties of tobacco for domestic consumption. Later during the Mahdi's rule, the use of tobacco in all its forms was considered a sin and thus prohibited. In the twentieth century, smoking of imported and locally manufactured cigarettes became increasingly popular among many Sudanese subjects.(5)

1.1.2 Prevalence

Despite increasing information about the adverse effects of smoking and bans on smoking by some governments, the use of tobacco continued to increase.(6) The third edition of the "Tobacco Atlas", published by the World Lung Foundation and the American Cancer Society, was launched in March 2009 during the 14th World Conference on Tobacco or Health in Mumbai, India. According to the atlas, more than one billion men and about 250 million women use tobacco every day.(7) The atlas states that, by 2010, tobacco will be killing 6 million people worldwide each year, and 72% of these deaths will be in low and middle-income countries. These countries are of particular focus for the tobacco companies because they have ineffective health policies and fewer resources to curb smoking.(7) It was estimated that since 1960 tobacco production has increased 300% in the developing countries while dropping more than 50% in the developed countries".(7) A 1988 WHO press release reported that while tobacco markets are decreasing in Western, industrialized countries at the rate of 1% per year, tobacco consumption is increasing in the developing countries at an average rate of 2% per year.(8)

In the Sudan, there is paucity of data regarding the prevalence of smoking among Sudanese subjects. In their cross sectional study in 1998 in the Nile State, Idris et al. found that 12% of adult males and 0.9% of adult females are cigarette smokers.(9) According to the study the prevalence of cigarette smoking is significantly higher in the urban areas whereas toombak use is higher in the rural areas.

In the past, the number of smokers rose as incomes increased within populations.(10) Smokers in high-income countries were more likely to be affluent than poor. This pattern appears to have reversed among men in the past few decades when affluent men have increasingly quit smoking, whereas poorer men have not.(10) A similar inverse relationship is found between educational levels and smoking. In general, individuals who have received little or no education are more likely to smoke than those who are more educated.(10) These two inverse relationships are unlikely to exist in the developing countries like the Sudan. A survey in Khartoum showed that 64% of doctors and university lecturers, and 34% of medical students, were smokers.(11) Similar results were obtained in other parts of the developing world.(12)

The 1998 prevalence of cigarette smoking among children and adolescents in the Nile State was quite low (about 2%).(9) Later in 2001, the Sudan Global Youth Tobacco Survey, a national school-based survey of pupils in 8^{th} primary grade "1^{st} secondary grade and 2^{nd} secondary grade" estimated that the use of all tobacco products by pupils was 20.3% in males and 12.9% in female.(13) In 2005, the survey was conducted again and the prevalence among school pupils of the same age group was 29.4% and 13.3% in males and females respectively.(14)

1.1.3 Contents of tobacco smoke

Tobacco smoke contains over 4000 chemicals in the form of particulates and gases.(15-19) The particulate phase (5% by weight) includes tar (itself composed of many chemicals), nicotine, benzene and benzo(a)pyrene. The gas phase (95% by weight) includes carbon monoxide, ammonia, dimethylnitrosamine, formaldehyde, hydrogen cyanide and acrolein. All these substances are emitted from a burning cigarette.(15-19) Some of these have marked irritant properties and some are known or suspected carcinogens. Tobacco smoke is classified as a class "A" carcinogen along with asbestos, arsenic, benzene and radon gas.(18,19)

1.1.4 Health hazards

As early as the eighteenth century, a number of physicians had observed a relation between the use of tobacco and lung disease. However, the relation to lung cancer remained largely debatable.(3) Around the middle of the twentieth century, several case control studies of lung cancer etiology were published in Europe and North America.(20-22) This led to a conclusion in 1950 that cigarette smoking is an important cause of lung cancer.(23) Since then, knowledge about the adverse health effects of smoking has accumulated. In 1964 a definitive proof that cigarette smoking was a serious health hazard was contained in a report by the Surgeon General's Advisory Committee on Health, appointed by the Public Health Service of the United States.(24) The committee drew evidence from numerous studies conducted over decades. They concluded that a smoker had a significantly greater chance of contracting lung cancer than a nonsmoker. The rate varied according to factors such as the number of cigarettes smoked per day, the number of years the subject smoked and the time in

the person's life when he or she began smoking.(24) Cigarette smoking was also found to be associated with cancers of the mouth, esophagus, nasopharynx, larynx, kidney, and urinary bladder,(25-28) as well as a cause of chronic obstructive pulmonary disease, impaired lung function, heart disease, stroke, and many other cardiovascular, pulmonary and neurological diseases.(29-34) In addition, it was an independent risk factor in male impotence.(35) On average, it was calculated that someone who smokes a pack or more of cigarettes each day lives 7 years less than someone who never smoked.(36)

1.2 Passive cigarette smoking

1.2.1 Background

Breathing other people's smoke is called passive, involuntary, environmental or secondhand smoking.(7) The environmental tobacco smoke is a major source of indoor air pollution. The non-smoker breathes "side-stream" smoke from the burning tip of the cigarette and "main-stream" smoke that has been inhaled and then exhaled by the smoker. Nearly 85% of the smoke in a room results from side-stream smoke and it is worth noting that many potentially toxic gases are present in higher concentrations in side-stream smoke than in main-stream smoke.(16,17)

1.2.2 Prevalence

The percentage of passive smokers in a population is far higher than that of active smokers. For example in the United States, where active smokers comprise approximately 26% of the adult population,(37) tobacco exposure is confirmed in more than 80% of non-smokers.(38) A bout 37% of adults

and 43% of children 2 months to 11 years of age live in homes with at least one smoker.(38) In the United Kingdom, almost half of all children are exposed to tobacco smoke at home.(39) It was estimated that almost half of the world's children were passive smokers.(7) In the Sudan, 28.4% of school pupils live in homes where others smoke in their presence and 16.5% of all pupils have one or more parents who smoke.(13)

1.2.3 Health hazards in adults

Evidence of the health impact of passive smoking has been building up over the past decades. The first conclusive evidence on the dangers of passive smoking is supposed to came from Takeshi Hirayama's study in 1981 on prevalence of lung cancer in non smoking Japanese women married to heavy smoker men.(40) Wives of heavy smokers were found to have a higher risk of developing lung cancer than wives of non-smokers. Since then, environmental tobacco smoke is increasingly recognized as a direct cause of disease in adults and children.

Some of the immediate effects of passive smoking include eye irritation, headache, cough, sore throat, dizziness and nausea.(41) Adults with asthma can experience a significant decline in lung functions when exposed to environmental tobacco smoke and increased bronchial reactivity.(42,43) However, significant increase in bronchial reactivity is not confirmed in Sudanese adults when exposed to environmental tobacco smoke before exercise.(44) In addition, short-term exposure has a measurable effect on the heart in non-smokers. Just 30 minutes exposure is enough to cause significant reduction in the coronary flow velocity reserve.(45)

In the longer term, passive smokers suffer an increased risk of a range of smoking-related diseases, including increased risk of respiratory disease, ischemic heart disease and lung cancer.(46-50) The 1992 United States Environmental Protection Agency review on passive smoking included reports on a relationship between environmental tobacco smoke, respiratory symptoms and sickness in adults.(51) Workplace exposure is more strongly related to respiratory symptoms than household exposure.(52-54) Studies conducted in some developed countries in the early 1990s estimated that heart disease caused by passive smoking was the third leading preventable cause of death, ranking behind active smoking and alcohol abuse, and that non-smokers living with smokers had an increased risk of heart disease of around 30%.(55-56) Another study in the United States showed an elevated risk of heart disease among passive smokers of around 20%.(57) In addition, Whincup et al. suggested that previous studies of the effects of passive smoking on the risk of heart disease might have been under-estimated. They found that blood cotinine levels, a biomarker of nicotine breakdown in the liver, among non-smokers were associated with a 50-60% increased risk of heart disease.(58) However, cotinine may not be specific for environmental tobacco exposure because dietary nicotine (eg, green pepper, tomato and tea) may elevate cotinine levels.

1.2.4 Health hazards in children:

Because many young children spend a large proportion of their time indoors, they may have significant exposure to environmental tobacco smoke at home.(59) This passive smoking has harmful effects on the respiratory health of such children.(60,61) Many studies have documented an increased incidence of lower respiratory tract infections in children

exposed to environmental tobacco smoke compared to those without exposure.(62-64) Infants whose parents smoke are more likely to have pneumonia and bronchitis than infants whose parents do not smoke.(65) Children with asthma whose parents smoke have more frequent exacerbations and more severe symptoms.(66-68) In an interventional study, it was demonstrated that if parents expose their children with asthma to less cigarette smoke, the asthmatic symptoms will be less severe.(69)

Middle ear problems that need surgical treatment are more likely to occur in children who live in households where more than three packs of cigarettes are smoked per day.(70) Middle ear effusion is about 60% more likely to develop in children whose parents smoke.(71) Moreover, a growing body of evidence links environmental tobacco smoke exposure to sudden infant death syndrome.(72-78) This relationship seems to be independent of birth weight and gestational age. Passive smoking has also been reported to alter lipid profiles in adolescents.(79) This may shed light on the mechanism of increased risk of coronary heart disease in passive smokers.

A number of studies have examined the relationship between passive smoking during childhood and the risk of cancer.(80-83) Some studies found some support for the hypothesis that paternal cigarette smoking is a potential risk factor for the generation of childhood cancers.(83) Exposure to tobacco smoke may also impair olfactory function in children. A Canadian study found that passive smoking reduced children's ability to detect a wide variety of odors compared with children raised in non-smoking households.(84) Passive smoking may also affect children's mental

development. A study conducted in USA found deficits in reading and reasoning skills among children even at low levels of smoke exposure.(85)

1.2.5 The adverse effects on pulmonary function tests in children

1.2.5.1 Pulmonary function tests

Pulmonary function tests are important tools for diagnosis and follow up of many respiratory problems. Abnormalities may appear even if the patient is asymptomatic. The tests assess the ventilatory functions of the lung (spirometry), airway resistance, gas diffusion, elastic properties of the lung, ventilation perfusion relationship and blood gases.(85)

Spirometry is the most commonly used test in assessment of lung ventilation. It detects presence of airflow obstruction or lung restriction, evaluates severity of obstruction or restriction, aids in the differential diagnosis of respiratory illness, assesses disease progression and evaluates response to treatment.(86) Spirometers are designed to measure changes in volume and when they are equipped with electronic signal outputs (pneumotachs), they also measure flow (volume per unit of time). The flow volume and volume time curves, as well as the absolute values for flows and volumes, should be considered during interpretation of spirometric results.

The parameters measured by spirometers include the following:

i- Forced vital capacity (FVC), the volume of air expired forcefully by full expiratory effort following maximum inspiration.

ii- Forced expiratory volume in the first second (FEV1), the volume of air expired in the first second of the FVC.

iii- FEV1/FVC ratio (FEV1/FVC%), the ratio of FEV_1 to FVC.

iv- The forced expiratory flow 25% (FEF25%), the amount of air that is forcibly expelled during the first 25% of the total forced vital capacity.

v- The forced expiratory flow 50% (FEF50%), the amount of air that is forcibly expelled during the first half of the total forced vital capacity.

vi- The forced expiratory flow 25-75% (FEF25 -75%), the amount of air expelled from the lungs during the middle half of the forced vital capacity.

vii- The peak expiratory flow rate (PEF), the highest speed of air expired by full expiratory effort following maximum inspiration.

The physiological basis of these tests depends on the development of a plateau of expiratory flow at any particular lung volume once a certain minimum expiratory pressure is achieved. The values obtained do not depend on the pressure applied, but on the mechanical characteristics of the lungs and airways.(87) A patient result is expressed in both an absolute number and a percentage of his normal predicted value.(88) Values of FEV1 and FVC that are over 80% of predicted are defined as within the normal range. Normally the FEV1 is about 80% of the FVC. In restrictive lung diseases like pulmonary fibrosis, both FEV1 and FVC are reduced, but characteristically the FEV1/ FVC ratio is normal or increased. In obstructive lung diseases such as asthma, the FEV1 is reduced much more than the FVC, giving a low FEV/FVC ratio.(89) The forced expiratory flow (FEF25 -75%) is closely related to FEV1 although occasionally it is reduced while FEV1 is normal. This is because it has the advantage of avoiding measurement during the effort dependent first quarter of the FVC.

Both FEV1 and FEF25-75% are reduced by an increase in airway resistance or decrease in elastic recoil of the lung. When spirometry is not available, the peak expiratory flow rate (PEF) can be measured by the peak flow meter. This is a portable, simple and cheap device that can easily be used by children. PEF provides simple quantitative measure of airflow obstruction. It has been used together with FEV1 ever since 1940.(90) Clinical guidelines on asthma management including the British Thoracic Society Guidelines (BTS) and the Guidelines of the National Asthma Education and Prevention Program of the United States recommend measurement of peak expiratory flow rate as part of the routine management of a patient with an exacerbation of asthma.(91,92)

Spirometric tests can be performed in children from age 6 year. Patience is required, particularly in young and inexperienced children, to obtain satisfactory FVC maneuvers; however, most children from age 9 years and above met adult-based American Thoracic Society (ATS) goals for spirometry test performance.(93) These goals include criteria for acceptability and reproducibility (mentioned in the next chapter).

1.2.5.2 The Adverse effects on passive smoking on lung functions

A growing body of scientific evidence indicates that childhood exposure to environmental tobacco smoke adversely affects lung function.(60,94-98) Several studies suggested that pulmonary function decrement in school-aged children was a result of combined early life (including intra-uterine life) and current exposure to parental smoking, especially the maternal smoking.(99-106) However, the negative effect of passive smoking on lung function is amplified in children with residual lung insult due to asthma,

cystic fibrosis or other lung disease.(107,108) Intra-uterine exposure to maternal smoking was associated with a large deficit in lung functions in children with asthma. This deficit was found to be independent of the effects of postnatal environmental tobacco smoke exposure.(107) Occasional low level of exposure to cigarette smoke seems to be associated with lung function alterations in adolescents.(109) On the other hand, some studies reported that intra-uterine exposure had no effect, suggesting that passive smoking after birth represents a major contributing factor to development and persistence of airflow obstruction or respiratory symptoms.(110,111)

1.3 Pathophysiology

1.3.1 Pathophysiology of respiratory diseases

Cigarette smoke contains over 4000 compounds, many of which are extremely reactive affecting the physiology of the respiratory system, cardiovascular system and other systems in the body.(15-19) The physiological responses to these chemicals in a passive smoker are generally the same as those in the active smoker but with a diminished effect.

Nicotine, a weak alkaloid consisting of a pyridine and a pyrrolidine rings, is the most addictive substance in cigarette smoke and thus sets the stage for continued exposure to the other constituents.(112) It meets all the criteria that define an addictive substance: it produces brief pleasurable psychoactive effects, its use occurs despite the known harmful effects, tolerance to both the pleasurable and unpleasant effects develops during early usage, higher doses overcome tolerance, and withdrawal symptoms

occur when the substance is no longer used.(112) Droplets of tar containing nicotine are deposited from cigarette smoke in the small airways and alveoli in the lungs where nicotine is buffered to physiological pH and is rapidly absorbed into the systemic circulation to reach the brain in about 10 to 19 seconds.(113) Nicotine acts on cholinergic nicotinic receptors that are found in large numbers in the brain. They are also found in sympathetic ganglia, parasympathetic ganglia, neuro-muscular junction and adrenal medulla.(86) Nicotinic receptors are ligand-gated ion channels that are made up of multiple subunits encoded by different genes. In the brain, many of these receptors are located presynaptically on glutamine secreting neurons. However, most of the other receptors are postsynaptic. Stimulation of these receptors by acetylcholine or small amount of nicotine results in sodium influx which causes depolarizing potentials followed by action potentials.(86) Nicotine has many effects in the body, including a pronounced effect on the major stress hormones. It stimulates hypothalamic corticotropin-releasing factor, increases levels of endorphins, adrenocorticotropic hormone and arginine vasopressin in a dose-related manner, releases corticosteroids in proportion to plasma nicotine concentration, alters the bioavailability of dopamine and serotonin and causes a sharp increase in heart rate and blood pressure.(32,114,115)

The respiratory physiology is also affected by tar, carbon monoxide, nitrogen oxides and many other substances found in cigarette smoke. Tar is the particulate component of smoke without the water or alkaloid content. It contains many carcinogens including polynuclear aromatic hydrocarbons, n-nitrosamines and aromatic amines.(116) Carbon monoxide binds tightly to hemoglobin in red blood cells forming carboxyhemoglobin. This decreases the capacity of the red blood cells to carry oxygen to vital

organs resulting in anemic hypoxia.(117) Adults who smoke have higher hemoglobin concentrations, which are believed to be a compensatory mechanism caused by release of erythropoietin from the kidney in response to the carbon monoxide-induced hypoxia.(118) An increased 2,3-diphosphoglycerate concentration, which alters oxygen's affinity for hemoglobin, can be correlated with tobacco smoke in children's blood.(117,119) Nitrogen oxides and many other oxidants contribute to the inflammatory response induced by cigarette smoking.(120) It is clear that, both active and passive exposure to tobacco smoke have a deleterious effect on the lung and on oxygen transport in children and adults.

Inflammatory cells in cigarette smokers are found predominantly in the lower respiratory tract with marked increase in alveolar macrophages.(121) They are typically filled with pigmented debris that is probably derived from cigarette smoke and cells injured by the smoke. In addition, neutrophils are present within the airway lumen and in the periglandular areas.(121) It is likely that cigarette smoke contributes to chronic inflammation in the lungs of smokers by activating the epithelial cells that line the airways to release proinflammatory cytokines and activating alveolar macrophages and complement system, thus generating proinflammatory mediators in a cell-independent manner. This probably leads to recruitment and activation of the diverse inflammatory cells present in the lungs of smokers.(122,123) Inflammation of the airway epithelium results in increased mucus production, decreased ciliary movement, beat frequency and transport, increased mucosal permeability to allergens, associated with increased total and specific immunoglobulin E levels in the blood and increased blood eosinophil counts.(124,125)

1.3.2 Inflammatory markers

Inflammatory markers such as IL-1, IL-4, IL-6, IL-8, TNFα, IFNγ and CRP can provide information about the mechanism of the inflammatory reactions associated with many respiratory and cardiovascular diseases.(126-132) These inflammatory markers or cytokines are hormone-like molecules that act generally in a paracrine fashion, to regulate immune responses.(86) They are secreted not only by activated lymphocytes and macrophages, but also by endothelial cells, bronchial epithelial cells and other types of cells. The effects of these cytokines include activation of T lymphocytes and promotion of inflammation (IL-1), activation of lymphocytes, monocytes and IgE class switching (IL-4), activation of lymphocytes and production of acute phase proteins (IL-6), chemotaxis for neutrophils, basophils and T lymphocytes (IL-8) and promotion of inflammation (TNF-α).(86) In many case control studies, the level of inflammatory markers was measured in body fluids of smokers and non smokers. Many of these studies found higher levels of inflammatory markers in serum, plasma or broncho-alveolar fluid of active and passive smokers compared to non-smokers.(133-137) However, some studies did not find any difference in concentration of these cytokines between passive smokers and non-smokers.(138-139) This variation in the pro-inflammatory responses may be due to genetic factor polymorphism and it may explain why certain individuals are more susceptible to the negative effects of smoking more than others.(140)

1.3.2.1 C-Reactive Protein

C - reactive protein (CRP) is an acute phase glycoprotein produced exclusively in the liver, in response to inflammatory cytokines such as IL-

6.(141,142) Its molecular weight is 120-140 kD. Although its structure is distinct from the immunoglobulins, it shares with them many biological activities. For example, it activates complement,(143) acts as an opsonin (144) and participates in generation of cytokines that enhance inflammation.(128) Its normal concentration in serum is less than 5 mg/L. Its level rises significantly in infectious and non infectious inflammation, tissue damage and in the presence of malignant tumors. For this reason, quantitative measurement of CRP is increasingly used as a marker of inflammation and tissue necrosis.(145) Because its biological half-life is less than 24 hours, concentration of CRP decreases much faster than ESR; that is why it is useful in follow up of the inflammatory process and in monitoring the response to treatment.(145) Passive smokers who are exposed regularly to environmental tobacco smoke have significantly higher C-reactive protein levels in plasma (146-148) indicating an ongoing inflammatory process. Several studies now support a strong link between baseline elevations of CRP and future risk of coronary events.(130,149) However, Helmersson et al. found no difference in serum CRP between smokers and non-smokers.(150)

1.3.2.2 Interleukin 4

Interleukin 4 (IL-4) is a 14 kD protein containing 130 amino acid residues. It is a potent multifunctional cytokine produced by activated T lymphocytes, mast cells and basophils. It stimulates growth and differentiation of B lymphocytes and maintains viability of certain subsets of B & T lymphocytes. In addition, it exerts a variety of biological effects on many non-lymphoid cells including endothelial cells and fibroblasts. Cigarette smoking has been associated with increased serum levels of total IgE and an increased risk of developing allergic-like symptoms.(151,152)

IL-4 and interferon-gamma (IFN-gamma) have reciprocal roles in the regulation of IgE synthesis. It is found that IL-4 production by peripheral blood mononuclear cells of smokers is significantly higher than that of non-smokers.(152) However, IFN-gamma production is not increased. This imbalance, favoring IL-4 production, may be part of the mechanism responsible for the observed increases in serum IgE and allergic-like symptoms associated with cigarette smoking.(152)

1.3.2.3 Tumor Necrosis Factor-α

Tumor necrosis factor alpha (TNF-α) is an extremely potent peptide cytokine (17 kD) that serves as an endogenous mediator of inflammatory, immune and host defense functions.(153-154) Several substances originally described for their biological activities, e.g. cachectin, macrophage cytotoxin (MCT) and necrosin, have been identified later as TNF-α.(155,156) TNF is produced mainly by macrophages in response to many bacterial, viral and parasitic products.(157) (e.g. lipopolysaccharides), but it is produced also by other cell types in response to IL1.(158)

TNFα has a broad spectrum of biological activities.(159-161) It acts either independently or in conjunction with other cytokines like IL1.(162) A local increase in concentration of TNFα will cause the cardinal signs of inflammation to occur: heat, swelling, redness, and pain; prolonged exposure to low concentrations can result in cachexia whereas high concentrations of TNFα induce shock-like symptoms. Other effects include the following:

i. Stimulation of the hypothalamus to release corticotrpin releasing hormone (CRH)

ii. Fever

iii. Inhibition of appetite

iv. Stimulation of the liver to release the acute phase proteins like cRP

v. Stimulation of chemotaxis for neutrophils and facilitation of diapedisis

vi. Stimulation of macrophages to phagocytose microorganisms and release IL1 and prostaglandin E2 (PGE2)

Many studies confirmed that TNFα production and concentrations in body fluids were greater in smokers than in non-smokers.(163-165) However, some attempts to examine the effect of cigarette smoking on TNFα production reported insignificant results.(166,167) Recent studies have shown that TNFα plays an important role in the induction of the cigarette related COPD and in the maintenance of airway inflammation.(168,169)

1.4 Justification

Passive smoking is a cause of lung cancer and ischemic heart disease in adults and a cause of respiratory disease, cot death, middle ear disease and asthmatic attacks in children. Genetic factors appear to play a role in determining who will be affected more by smoking; in a comparative study of lung function in relation to passive smoking between American and French women, passive smoking was found to be significantly related to lower FVC and FEV_1 values among the French women but not the American ones.(140) In another study, the importance of cytokine genetic polymorphism in determination of who is going to develop cigarette induced chronic obstructive pulmonary disease was documented.(170)

Taking the dangerous hazards of active and passive smoking into consideration, the World Health Organization Framework Convention on Tobacco Control calls for nations to establish progressively a national system for the epidemiological surveillance of tobacco consumption and related social, economic and health indicators.(171) In this regard, the adverse health effects of tobacco products, especially toombak, on Sudanese subjects is well investigated.(172-183) The Department of Physiology has investigated the short term effects of tobacco smoke on the respiratory system in adults.(44,184) However, the effect of passive cigarette smoking on the respiratory system in school children and its relation to the level of inflammatory markers in the plasma is not covered.

1.5 Objectives

1.5.1 General objective

i. To determine the effects of passive smoking on spirometric values and plasma level of inflammatory markers in healthy male school-children.

1.5.2 Specific objectives

i. To compare results of spirometry between passive smokers and non-smokers by measuring ventilatory functions of the lung in healthy male school-children using a spirometer.

ii. To measure plasma level of the inflammatory markers (IL4, CRP and TNFα) in healthy male school-children in order to compare between passive smokers and non-smokers.

CHAPTER TWO

2. SUBJECTS AND METHODS

2.1 Study design

This is an analytic observational case control study.

2.2 Study area and population

The study was conducted in three governmental primary schools for boys in Khartoum (Arkweet Shamal in Arkweet, Elshaheed Muatasim Parakat in Imtidad Naser & Alimtidad 1 in Alimtidad Eldarga 3), between August 2006 and January 2009. The schools were selected randomly from 92 primary schools, the total number of public schools for boys in Khartoum. Each school contains 8 classes, in each class there are about 40 to 60 pupils; their ages range from 6 to 15 years old. All pupils in the three schools were approached. Inclusion and exclusion criteria were used to select the test and control groups. The selected pupils were 9 to 14 years old, in the fourth to the seventh grades. Parents of the pupils were generally of the average socio-economic class. They lived in houses near the schools of their children. Most of the fathers were manual workers or employees whereas the majority of the mothers were housewives.

2.3 Sample size

The sample size of each group (test and control groups) was calculated using the formula: $n > 2[(z_{2\alpha} + z_{2\beta})\sigma/\delta_1]^2$; where n= the sample size, z= the standardized deviate, α= type I error, β= type II error, σ= the standard deviation & δ= the true difference.(185) The calculated result for each group (test or control group) was 63. About 573 pupils were approached and only 135 of them fulfilled the inclusion criteria. Those included in the test group were 69 pupils whereas the other 66 pupils were included in the control group.

2.4 Inclusion and exclusion criteria

Inclusion criteria for the test group were: school-pupil, age 9 - 14 years, healthy with normal BMI, has no symptoms or signs of acute or chronic medical illness during the last four weeks, no signs of respiratory disease or chest deformity, not on medical treatment, not active smoker, living with at least one smoker at home, exposed regularly to cigarette smoke at home of not less than two cigarettes per day for most days, since birth and not regularly exposed to environmental tobacco smoke or any other type of smoke outside the house.

Inclusion criteria for the control group were: school-pupil, age 9 - 14 years, healthy with normal BMI, has no symptoms or signs of acute or chronic medical illness during the last four weeks, no signs of respiratory disease or chest deformity, not on medical treatment, not active smoker and not exposed to environmental tobacco smoke or any other type of smoke inside or outside the house.

Exclusion criteria for both groups were: age less than 9 or more than 14 years, abnormal BMI, presence of symptoms of acute or chronic medical illness during the last four weeks, presence of abnormal chest signs or skeletal deformity on clinical examination, being on medical treatment, active smoker, exposed to cigarette smoke or any other type of smoke inside or outside the house (for the group of controls) or exposed to smoke of less than two cigarettes per day inside the house (for the group of cases).

Both groups were matched according to age, sex, height, weight and area of residence. The nutritional status (assessed by BMI) of the two groups was also considered.

2.5 Methods

2.5.1 Questionnaire

Four "interviewer administered questionnaire papers" were prepared (see appendix). Data required by the questionnaire papers were collected from each pupil and his parents as follows:

i. Information collected from the pupil was about his age, class, school performance, health problems, smoking habits, exposure to environmental cigarette smoke at home or outside the house, frequency of exposure, exposure to any other type of smoke in or outside the house, number of smokers in the house and who is smoking.

ii. Information collected from the father of the pupil was about the father's age, tribe, address, educational attainment, job, health insurance, smoking habits, his knowledge about the adverse health effects of passive smoking and his attitude and behavior towards smoking at home, near his children or near his wife during pregnancy.

iii. Information collected from the mother of the pupil was about the mother's age, address, educational attainment, job, smoking habits, her knowledge about the adverse health effects of passive smoking and her attitude and behavior towards smoking at home, near her children or during pregnancy.

iv. Information about the pupil's health status was collected from the parents. It included information about the present health status of their child, wheezes, allergy, snoring at sleep, past history of hospital admissions, surgical operations and history of infections like pneumonia, influenza and otitis media during the last year. The questionnaire paper also requested a signed permission to take a blood sample and to test the lung function of their child using a spirometer.

2.5.2 Evaluation of the degree of exposure

The information obtained from each pupil and his family about the degree of exposure to cigarette smoke at home was used for classification of the pupils into three subgroups: group of no exposure (includes all the controls), group of mild-moderate exposure (exposed to smoke of 2 to 5 cigarettes/day) and group of heavy exposure (exposed to smoke of > 5 cigarettes/day).

2.5.3 Clinical examination:

The selected pupil was examined clinically to exclude presence of any abnormal clinical sign that might interfere with the normal function of his respiratory system such as pleural effusion, pneumothorax, asthma and signs of chest deformity or skeletal abnormality. The height and weight of each pupil were measured using standardized height and weight scales. The Body mass index (BMI) was calculated for each pupil as weight (in kilograms)/height (in meters^2). The BMI results were compared to normal values of BMI adjusted for age and sex.(218)

2.5.4 Spirometry

A portable spirometer (Clement Clarke All-flow Spirometer) was used to measure FVC, FEV_1, FEF_{50} and PEF for each pupil. Measurements were carried out according to the guidelines of the American Thoracic Society.(186) These guidelines provide criteria for standardization of spirometry performance, including criteria for acceptability and reproducibility of the test results.

2.5.4.1 Criteria for acceptability:

i. Lack of artifact induced by coughing, glottis closure or equipment problems

ii. Satisfactory start to the test without hesitation

iii. Satisfactory exhalation with six seconds of smooth continuous exhalation and/or a plateau in the volume time curve of at least one second, or a reasonable duration of exhalation with a plateau.

2.5.4.2 Criteria for reproducibility (after three acceptable results):

i. The largest FVC should be within 0.2 L of next largest FVC

ii. The largest FEV_1 should be within 0.2 L of next largest FEV_1

iii. If these two criteria are not met, additional spirograms should be obtained up to eight efforts.

iv. The largest values of FVC, FEV_1, FEF_{50} and PEF are reported.

2.5.4.3 Measurement steps:

i. Air temperature and relative humidity of the room were measured and registered in a computer program that controls the spirometer.

ii. The spirometer was calibrated with a 3L calibrating syringe.

iii. The age, sex, height and weight of each pupil were registered in the computer program.

iv. Each pupil was asked to stand up, clip his nose with a nose clip, take a deep breath, put a mouthpiece connected to the spirometer in his mouth with the lips tightly around it, and then blow air out as hard and as fast as possible for at least 6 seconds.

v. The procedure was repeated according to the ATS criteria (mentioned above) until acceptable and reproducible results were obtained.

vi. Total measurement trials for each pupil did not exceed eight times to avoid exhaustion.

2.5.5 Collection of blood Samples

Chemically clean and sterile disposable needles, syringes and swabs were used for all blood samples. About 5 ml of blood was withdrawn from each pupil from the antecubital vein. The blood was collected in a tube containing EDTA as an anticoagulant. Then the tube was sealed, labeled with the pupil's number, incubated in ice and sent immediately to the laboratory for full blood counts and plasma separation.

2.5.6 Plasma separation and storage

Plasma was prepared with centrifugation within two hours of venipuncture. Plasma samples were frozen and stored at -20 ^{o}C for biochemical analysis.

2.5.7 Technique of biochemical analysis

2.5.7.1 General principles

The inflammatory markers IL4, TNFα and CRP were measured using commercially available quantitative sandwich enzyme-linked immuno-sorbent assay (ELISA) kits according to instructions of the manufacturers. The analyses were performed with 96-well microtiter plate ELISA kits for IL4 & TNFα (from BD Biosciences Pharmingen/USA), and NycoCard Reader II quantitative test for CRP (from Axis-Shield/Norway). Test sensitivity was 2 pg/ml for IL4, 2 pg/ml for TNF-α, and 5 mg/L for CRP. Microtiter strips pre-coated with monoclonal antibodies generated against the proteins were used for quantification.

2.5.7.2 ELISA Procedure

i. 50 µL ELISA diluent (a buffered protein base with 0.09% sodium azide as preservative) was added to each well.
ii. 100 µL standard (animal serum with 0.09% sodium azide as preservative) or sample was added to each well and incubated for two hours at room temperature.
iii. The mixture was aspirated and washed with concentrated detergent solution for 5 times.
iv. 100 µL prepared working detector (biotin-conjugated antibody) was added to each well and incubated for one hour at room temperature.
v. The mixture was aspirated and washed with concentrated detergent solution for 7 times.
vi. 100 µL TMB One-Step Substrate Reagent (3,3',5,5'-tetramethylbenzidine in a buffered solution) was added to each well and incubated for 30 minutes at room temperature.
vii. 50 µL stopping solution (1 M phosphoric acid) was added to each well and the intensity of the color was measured at 450 nm within 30 minutes.

2.6 Ethical consideration

The research conforms to the ethical principles of medical research developed by the World Medical Association Declaration of Helsinki.(187) Ethical approval was given by the Research Committee (Faculty of Medicine/ University of Khartoum). Approval was obtained from the Unit of School Health/ Ministry of Education. Permission letters were submitted to the headmasters of the selected schools. Written consents were obtained from the parents before entry into the study.

2.7 Statistical analysis

All data obtained with questionnaire, spirometry and biochemical analysis was analyzed using the Statistical Package for the Social Sciences (SPSS) version 14. The chi square test was used to test distribution of categorical variables and student's t test was used for continuous variables. The difference between the test and the control groups were assessed with the student's t test. Statistical significance was accepted when P value is less than 0.05.

2.8 Data presentation

Final results were presented in tables and figures.

2.9 Declaration

Almost all phases of this research and most technical methods of data collection were done by me. These include obtaining the ethical approval from the educational authorities, coverage of half of expenses of training courses on spirometric measurements, standardization and interpretation at Royal Brompton Hospital in London/ United Kingdom, courses on statistical analysis using the SPSS program, random selection of the schools and the pupils, questionnaire application, collection of information from each pupil and his parents, clinical examination, BMI measurements, lung function test measurements, plasma separation and storage and statistical analysis; however, the technical parts that need special experience were done by trained laboratory technicians during my attendance and under my supervision. These include the process of blood collection from the pupils and the biochemical analysis.

CHAPTER THREE

3. RESULTS

3.1 Demographic characteristics of the study group

3.1.1 Age distribution

The ages of school pupils in the study group ranged between 9 and 14 years old with a mean of 11.6 ± 1.3 year (mean ± SD). (Fig. 1)

The mean age for the group of cases was 11.7 ± 1.3 year and for the group of controls was 11.5 ± 1.2 year. There was no significant statistical difference in age distribution between the two groups. (Table 1), (Fig. 2)

3.1.2 Height distribution

The height of school pupils in the study group ranged between 121 and 170 cm, with a mean of 144.13 ± 9.00 cm. It showed normal distribution curve. (Fig. 3)

The mean height for the group of cases was 144.25 ± 9.85 cm and for the group of controls was 144.00 ± 8.19 cm. There was no significant statistical difference in height distribution between the two groups. (Table 1), (Fig. 4)

3.1.3 Weight distribution

The weight of school pupils ranged between 21 and 50 kg, with a mean of 34.79 ± 6.28 kg. (Fig. 5)

The mean weight for the group of cases was 34.99 ± 6.83 kg and for the group of controls was 34.58 ± 6.69 kg. There was no significant

statistical difference in weight distribution between the two groups. (Table 1)

3.1.4 Body mass index (BMI) distribution:

The BMI for pupils in the study group ranged between 13.92 and 21.7, with a mean of 16.63 ± 1.72.

The mean BMI for the group of cases was 16.66 ± 1.72 and for the group of controls was 16.60 ± 1.73. There was no significant statistical difference in BMI distribution between the two groups. (Table 1)

3.1.5 Blood cells, hemoglobin & red blood cell indices

Complete blood count showed the following values in the study group: mean WBC count was 6.42 ± 2.24 /µL, mean RBC count was 4.75 ± 0.49 /µL, mean hemoglobin concentration was 11.9 ± 1.10 g/dL and mean packed cell volume was 35.95 ± 3.25%.

Statistical analysis did not show significant statistical difference in the results of complete blood count between the group of cases and that of controls. (Table 2)

3.1.6 Sources of second-hand smoke in the study group

Fathers were responsible for 67% of passive smoking in the group of cases. Mothers were responsible for about 2% whereas relatives like brothers and uncles were responsible for about 30%. (Fig. 6)

3.2 Effect of passive smoking on plasma level of inflammatory markers

3.2.1 Effect on plasma CRP

The mean plasma CRP was 1.13 ± 0.34 mg/L (mean ± SEM) in the group of cases and 0.47 ± 0.25 mg/L in the group of controls. Although the mean was higher in the group of cases than in the group of controls, the difference was not statistically significant. (Table 3)

The mean plasma CRP was 0.47 ± 0.25 mg/L in those who were not exposed to cigarette smoke (group of no exposure), 1.04 ± 0.36 mg/L in the group of mild-moderate degree of exposure and 1.32 ± 0.72 mg/L in the group of high degree of exposure. The level was higher in those with mild-moderate exposure than those with no exposure; and highest in those with high degree of exposure. The difference between the three groups was not statistically significant. (Table 4)

3.2.2 Effect on plasma IL4

The mean plasma IL4 was 2.92 ± 0.93 pg/ml in the group of cases and 0.45 ± 0.28 pg/ml in the group of controls. The difference between the two groups was statistically significant ($P < 0.05$). (Table 3)

When the degree of exposure was considered, the mean plasma IL4 was lowest (0.45 ± 0.28 pg/ml) in those who were not exposed to cigarette smoke, higher (2.72 ± 1.24 pg/ml) in those with mild-moderate degree of exposure and highest (3.33 ± 1.24 pg/ml) in those with high degree of exposure. The difference between the three groups was statistically significant ($P < 0.05$). (Table 4)

3.2.3 Effect on plasma TNF-α

The mean plasma TNFα was higher in the group of cases (19.78 ± 4.67 pg/ml) than the group of controls (5.05 ± 1.54 pg/ml). This difference was statistically significant ($P < 0.05$). (Table 3)

The mean plasma TNFα was 5.05 ± 1.54 pg/ml in those who were not exposed to cigarette smoke, 12.42 ± 3.23 pg/ml in those with mild-moderate degree of exposure and 35.50 ± 12.47 pg/ml in those with high degree of exposure. The level was higher in those with mild-moderate exposure and highest in those with high degree of exposure. The difference between the three groups was found to be statistically significant ($P < 0.05$). (Table 4)

3.3 Effect of passive smoking on spirometric values

3.3.1 Effect on FVC

The mean FVC was 2.41 ± 0.35 L (mean ± SD) in the group of controls and 2.21 ± 0.57 L in the group of cases (reduction by about 8%). The difference between the means of the two groups was statistically significant ($P < 0.05$). (Table 5)

The mean FVC was highest (2.41 ± 0.35 L) in those who were not exposed to cigarette smoke, lower (2.24 ± 0.60 L) in those with mild-moderate degree of exposure and lowest (2.15 ± 0.51 L) in those with high degree of exposure to cigarette smoke. However, the difference between the three groups was not statistically significant. (Table 6)

3.3.2 Effect on FEV_1

The mean FEV_1 was lower in the group of cases (2.03 ± 0.46 L) than the group of controls (2.20 ± 0.42 L) by about 7%. This difference was found to be statistically significant ($P < 0.05$). (Table 5)

When the degree of exposure was considered, the mean FEV_1 was 2.20 ± 0.41 L in those who were not exposed to cigarette smoke, 2.07 ± 0.47 L in those with mild-moderate degree of exposure and 1.95 ± 0.42 L in those with high degree of exposure. Although there is progressive reduction in the value of FEV_1, showing a dose related response to cigarette smoke exposure; the difference between the three groups was not statistically significant. (Table 6)

3.3.3 Effect on FEF_{50}

The mean FEF_{50} was 3.24 ± 0.77 L/s in all school pupils of the study group, 3.20 ± 0.73 L/s in cases and 3.28 ± 0.81 L/s in controls. The mean was lower in the group of cases than in controls; however, this difference was not statistically significant. (Table 5)

When the degree of tobacco smoke exposure was considered, the mean FEF_{50} was 3.27 ± 0.81 L/s in those who were not exposed to cigarette smoke, 3.29 ± 0.76 L/s in those with mild-moderate degree of exposure and 3.02 ± 0.66 L/s in those with high degree of exposure. The mean is lower in those with high degree of exposure than those without or with mild-moderate degree of exposure. However, the difference was not statistically significant. (Table 6)

3.2.4 Effect on PEF

The mean PEF was 4.42 ± 0.97 L/s in the group of cases and 4.54 ± 0.63 L/s in the group of controls. It is lower in the group of cases than the group of controls; however, this difference was not statistically significant. (Table 4)

The mean PEF was 4.54 ± 0.63 L/s in those who were not exposed to cigarette smoke, 4.41 ± 1.07 L/s in those with mild-moderate degree of exposure and 4.45 ± 0.76 L/s in those with high degree of exposure. The difference between the three groups was not statistically significant. (Table 6)

3.4 Knowledge and attitude of parents towards passive smoking

3.4.1 Educational attainment of fathers in the study group

About 21% of all fathers were primary school graduates, 12% were intermediate school graduates, 39% were higher secondary school graduates, 17% were university graduates and about 10% were non-educated. The percentage of passive smoking was low in children of non-educated and university graduate fathers, and high among children whose fathers received primary, intermediate secondary or higher secondary school education. The difference in educational attainment between fathers of the group of cases and fathers of the group of controls was not statistically significant. (Fig. 7)

3.4.2 Educational attainment of mothers in the study group

About 21% of all mothers completed primary school education, 15% completed intermediate secondary school education, 34% completed

higher secondary school education, 15% were university graduates and about 15% were non-educated. The percentage of passive smoking was low in children of non-educated mothers or those who received higher secondary school education. It was higher among children whose mothers received primary, intermediate secondary or university education. However, the difference in educational attainment between mothers of the two groups (cases & controls) was not statistically significant. (Fig. 8)

3.4.3 Knowledge about adverse effects of passive smoking

The majority of parents in the study group (82%) believed that passive smoking is associated with complications and adverse effects on child's health, with slightly higher percentage for the parents of controls (88% of all controls) than the parents of cases (75% of all cases); however, this difference was not statistically significant. (Fig. 9)

3.4.4 Attitude of fathers towards smoking near their wives during pregnancy

All fathers of the pupils in the group of controls and the majority (90%) of fathers of the pupils in the group of cases did not smoke or allow relatives to smoke near their wives during pregnancy. The remaining 10% of all fathers in the study group smoked near their wives during pregnancy. The difference between fathers of the group of cases and fathers of the group of controls was statistically significant ($P < 0.05$). (Fig. 10)

3.5 Effect of passive smoking on child's health and school performance

3.5.1 Hospital admissions among cases and controls

About 21% of all pupils in the study group had positive past history of hospital admission for more than 24 hours. About half of these pupils (10.37%) were in the group of cases and the other half were in the group of controls. There was no significant statistical difference between the two groups. (Fig. 11)

3.5.2 Surgical operations among cases and controls

About 9% of all pupils in the study group had a past history of surgical operation. About two thirds were from the group of cases and the rest were from the group of controls. Although the percentage of operations was higher in the group of cases than the group of controls, statistical tests did not show any significant difference between the two groups. (Fig. 12)

3.5.3 Childhood illnesses among cases and controls

The percentage of pupils who had influenza like illness during the last year was higher in the group of cases (14.8%) than the group of controls (9.6%). However, this difference was not statistically significant. (Fig. 13)

The percentage of pupils who had pneumonia was higher in the group of cases (6.7%) than the group of controls (3.7%). However, no significant statistical difference was found between the two groups. (Fig. 13)

Positive past history of otitis media was higher in the group of cases (2.2%) than the group of controls (1.5%). However, the difference between the two groups was not statistically significant. (Fig. 13)

3.5.4 Snoring among cases and controls

Snoring during sleep was higher in the group of cases (8.2%) than the group of controls (2.2%). Statistical tests show significant difference between the two groups ($P < 0.05$). (Fig. 14)

3.5.5 School performance among cases and controls

The percentage of pupils with excellent academic performance in the last school examination was (5.9%) in the group of cases and (9.6%) in the group of controls. On the other hand, the percentage of pupils with weak performance was higher in the group of cases (20.0%) than the group of controls (15.6%). However, the difference between the two groups was not statistically significant. (Fig. 15)

Table 1: Age, Height, Weight & Body Mass Index among Cases & Controls in the Study Group

	Cases (n = 69)			Controls (n = 66)		
Parameter	Mean ± SD	Min	Max	Mean ± SD	Min	Max
Age (y)	11.70 ± 1.34	9.0	14.0	11.53 ± 1.2	9.0	14.0
Height (cm)	144.24 ± 9.85	121	170	144.00 ± 8.19	128	166
Weight (Kg)	34.99 ± 6.83	21	50	34.58 ± 5.69	25	50
BMI	16.66 ± 1.72	13.92	21.36	16.60 ± 1.73	14.18	21.7

Table 2: Mean White Blood Cell Count, Red Blood Cell Count, Hemoglobin Concentration & Packed Cell Volume among Cases & Controls in the Study Group

Parameter	Cases (n = 69) Mean ± SD	Controls (n = 66) Mean ± SD	Normal value*
TWBC ($x10^3$ cell/μL)	6.39 ± 2.26	6.45 ± 2.23	4.50-13.50
RBC ($x10^6$ cell/μL)	4.75 ± 0.48	4.74 ± 0.49	3.8-5.5
Hb (g/dL)	11.97 ± 0.99	11.83 ± 1.2	11-16
PCV (%)	36.33 ± 3.33	35.56 ± 3.16	34-40

** Source: Nelson Essentials of Pediatrics (5^{th} edition)*

Table 3: Mean Plasma Levels of Inflammatory Markers among Cases & Controls in the Study Group

Parameter	Cases (n = 69) Mean ± SEM	Controls (n =66) Mean ± SEM
CRP (mg/L)	1.13 ± 0.34	0.47 ± 0.25
IL4 (pg/ml)	2.92 ± 0.93*	0.45 ± 0.28
TNFα (pg/ml)	19.78 ± 4.67*	5.05 ± 1.54

$P^* < 0.05$

Table 4: Plasma Levels of Inflammatory Markers According to Degree of Cigarette Smoke Exposure (Mean ± SEM)

Parameter	No exposure (n=66) Mean ± SEM	Mild-moderate exposure (n=47) Mean ± SEM	High exposure (n=22) Mean ± SEM
cRP (mg/L)	0.47 ± 0.25	1.04 ± 0.36	1.32 ± 0.72
IL4 (pg/ml)	0.45 ± 0.28	2.72 ± 1.24*	3.33 ± 1.24*
TNFα (pg/ml)	5.05 ± 1.54	12.42 ± 3.23*	35.50 ± 12.47*

$P^* < 0.05$

Table 5: Distribution of Mean Spirometric Values among Cases & Controls in the Study Group

	Cases	Controls
Parameter	Mean ± SD	Mean ± SD
FVC (L)	2.21 ± 0.57*	2.41 ± 0.35
FEV_1 (L)	2.03 ± 0.46*	2.20 ± 0.42
FEF50 (L/s)	3.20 ± 0.73	3.28 ± 0.81
PEF (L/s)	4.42 ± 0.97	4.54 ± 0.63

$P^* < 0.05$

Table 6: Distribution of Mean Spirometric Values According to Degree of Cigarette Smoke Exposure

Parameter	No exposure (n=66) Mean ± SD	Mild-moderate exposure (n=47) Mean ± SD	High exposure (n=22) Mean ± SD
FVC (L)	2.41 ± 0.35	2.24 ± 0.60	2.15 ± 0.51
FEV_1 (L)	2.20 ± 0.41	2.07 ± 0.47	1.95 ± 0.42
FEF_{50} (L/s)	3.27 ± 0.81	3.29 ± 0.76	3.02 ± 0.66
PEF (L/s)	4.54 ± 0.63	4.41 ± 1.07	4.45 ± 0.76

Figure 1: Age Distribution in the Study Group

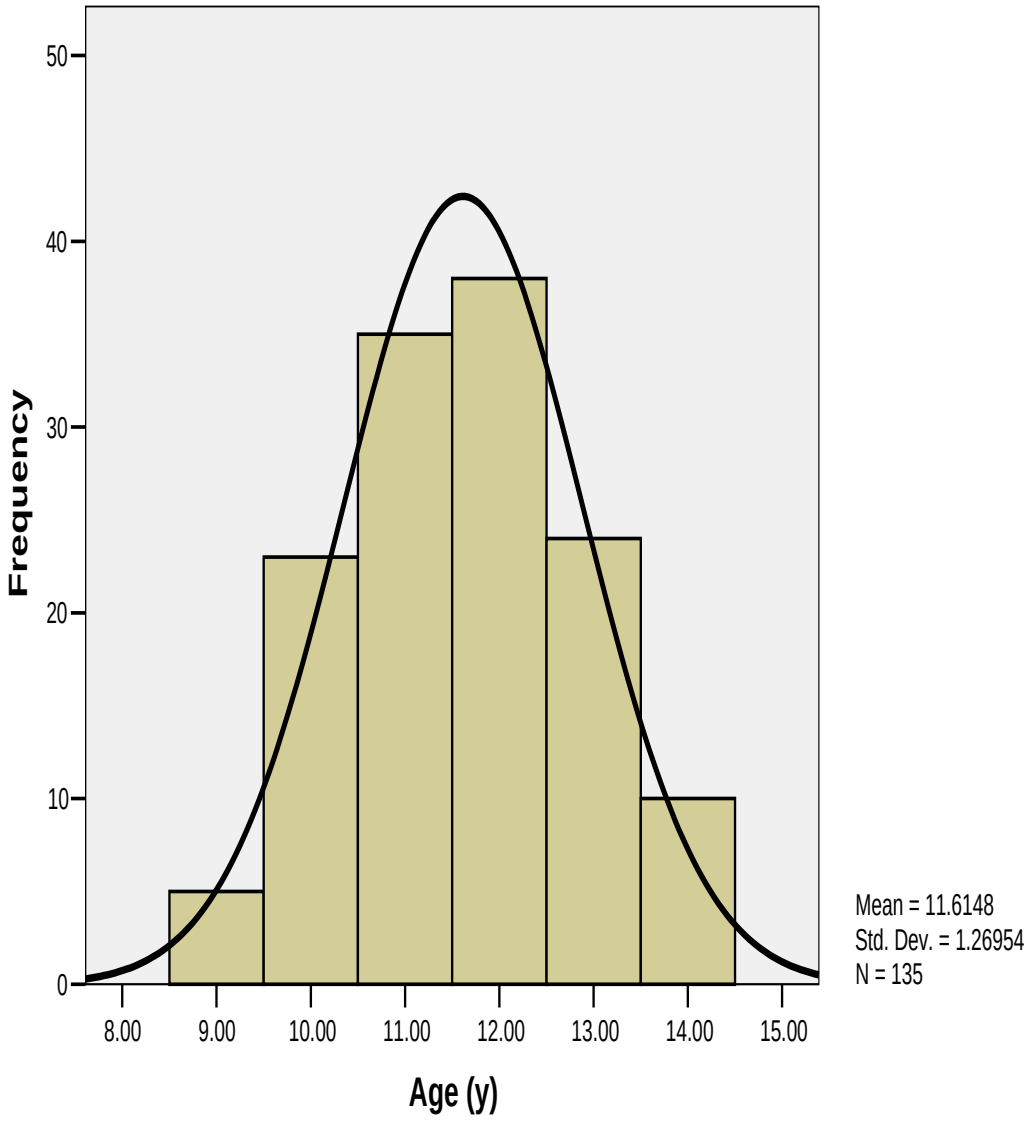


Figure 2: Age Distribution among Cases and Controls in the Study Group

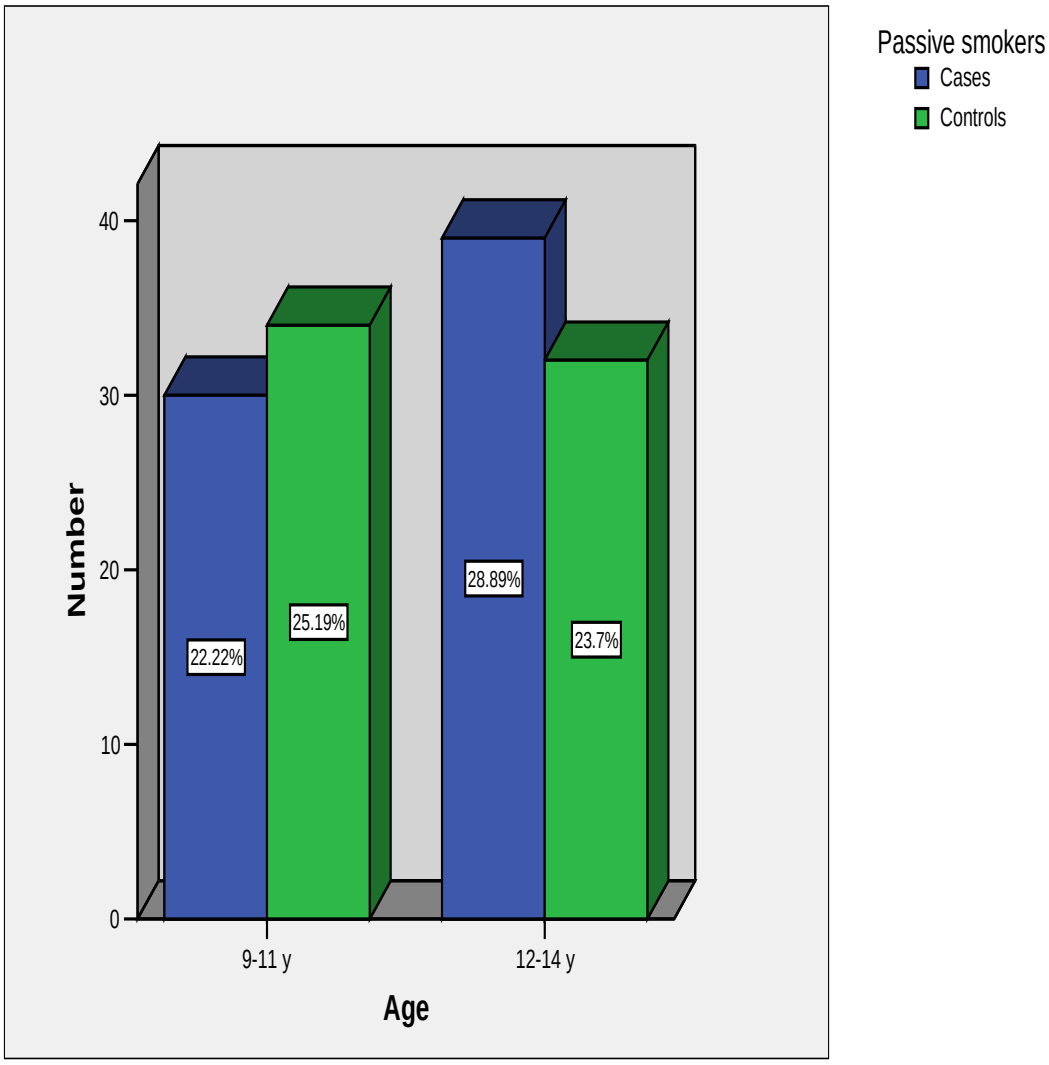


Figure 3: Height Distribution in the Study Group

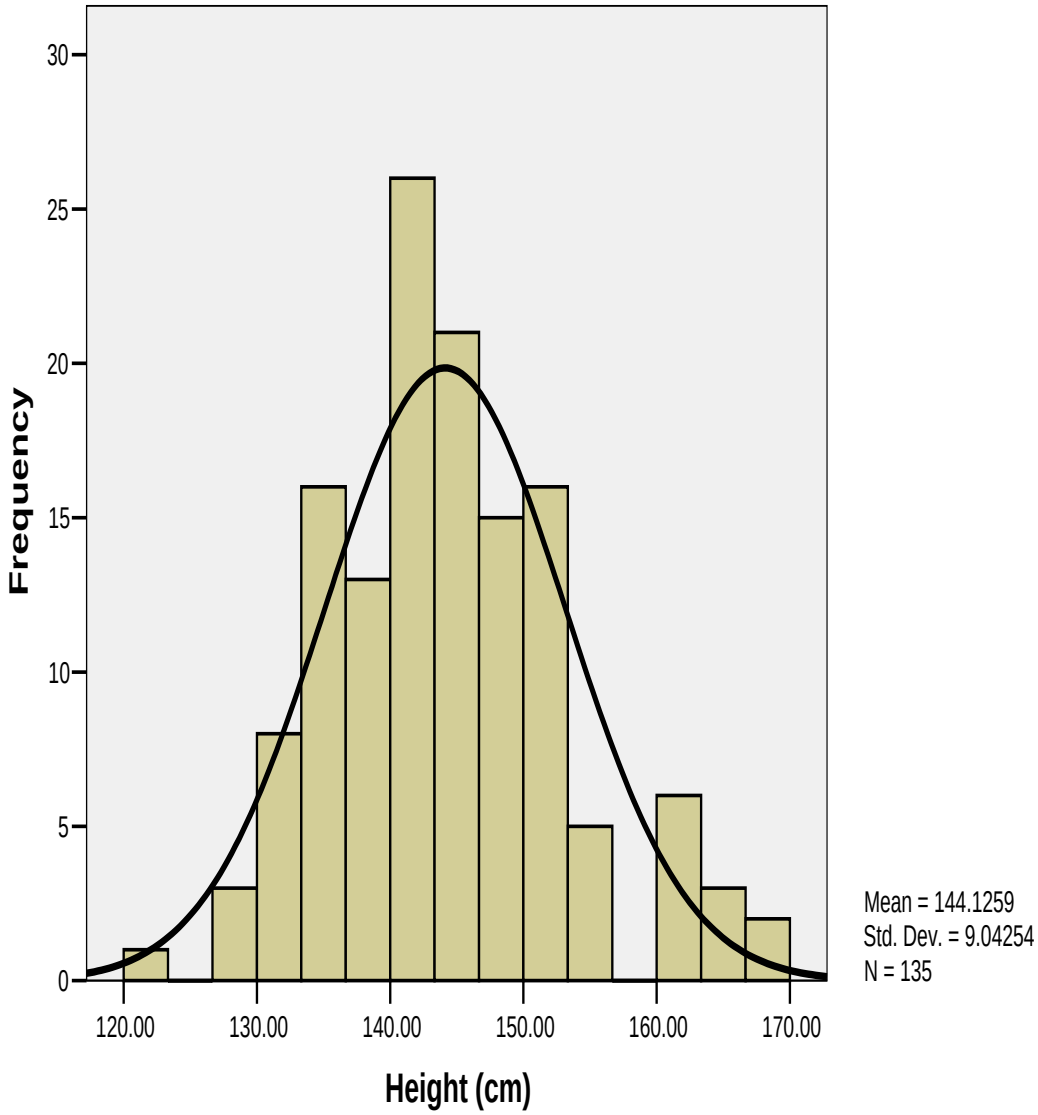


Figure 4: Height Distribution among Cases and Controls in the Study Group

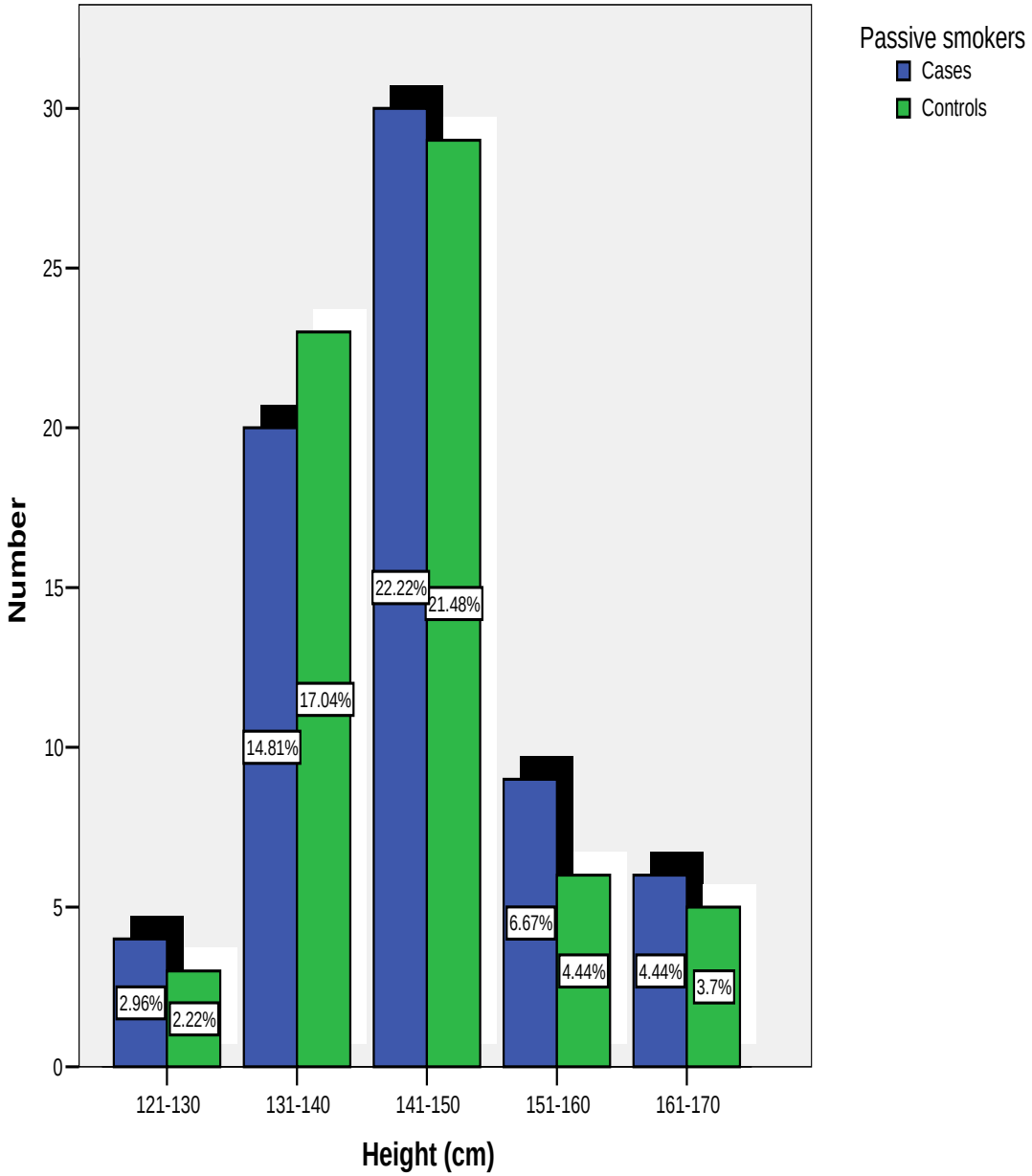


Figure 5: Weight Distribution among Cases and Controls in the Study Group

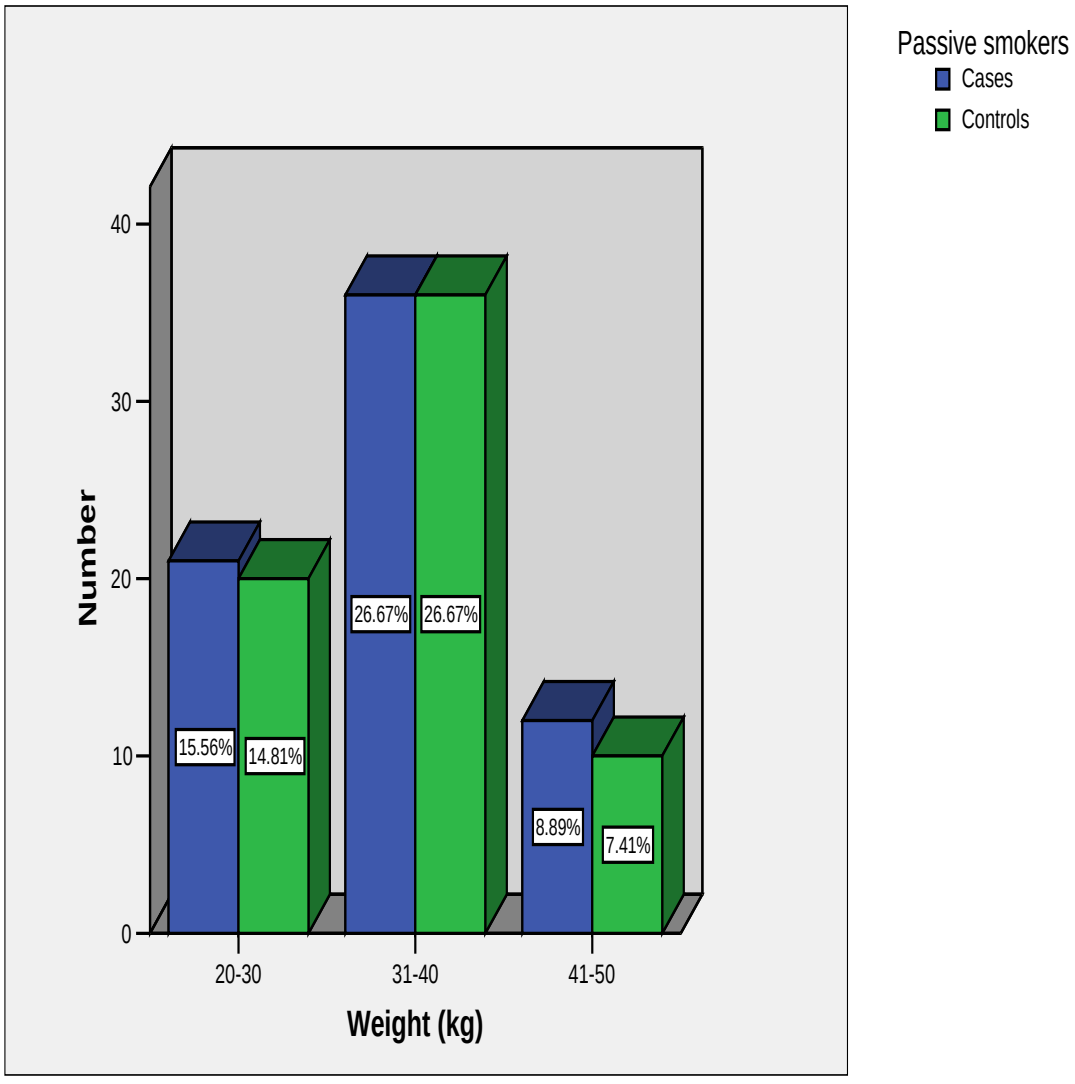


Figure 6: Sources of Second Hand Smoke in the Study Group

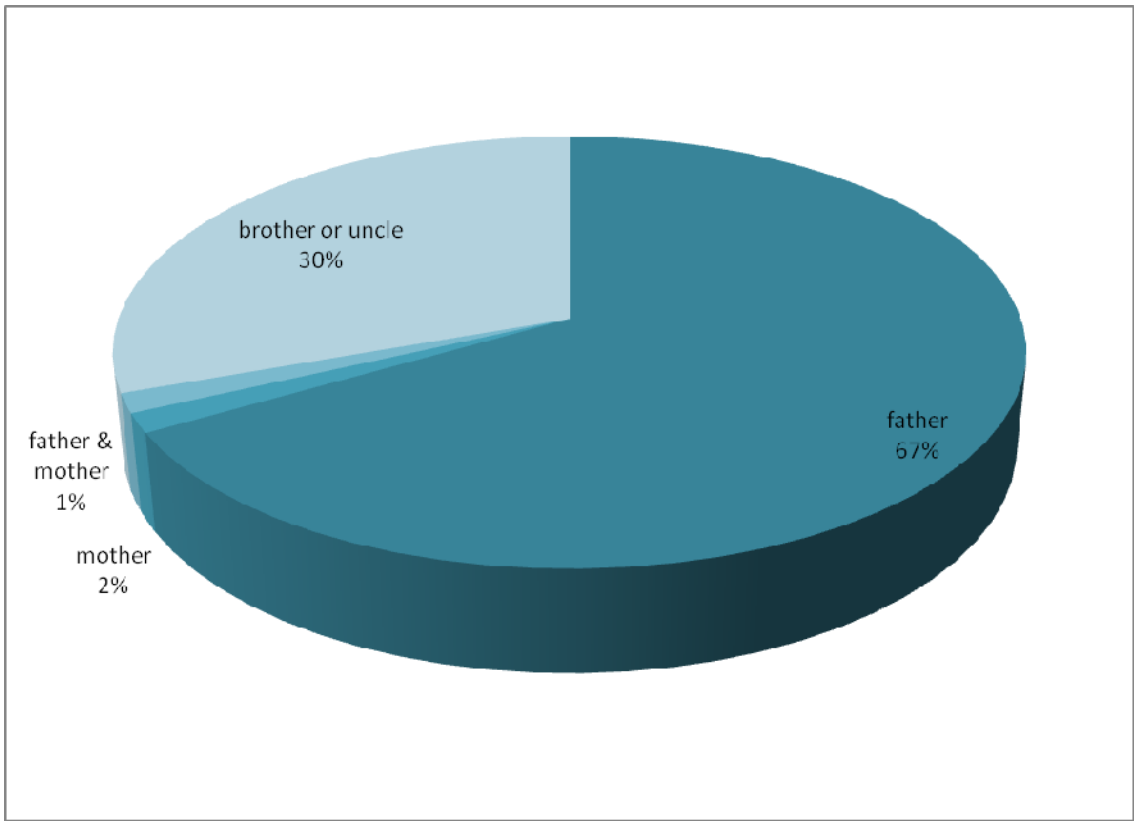


Figure 7: Educational Attainment of Fathers in the Study Group

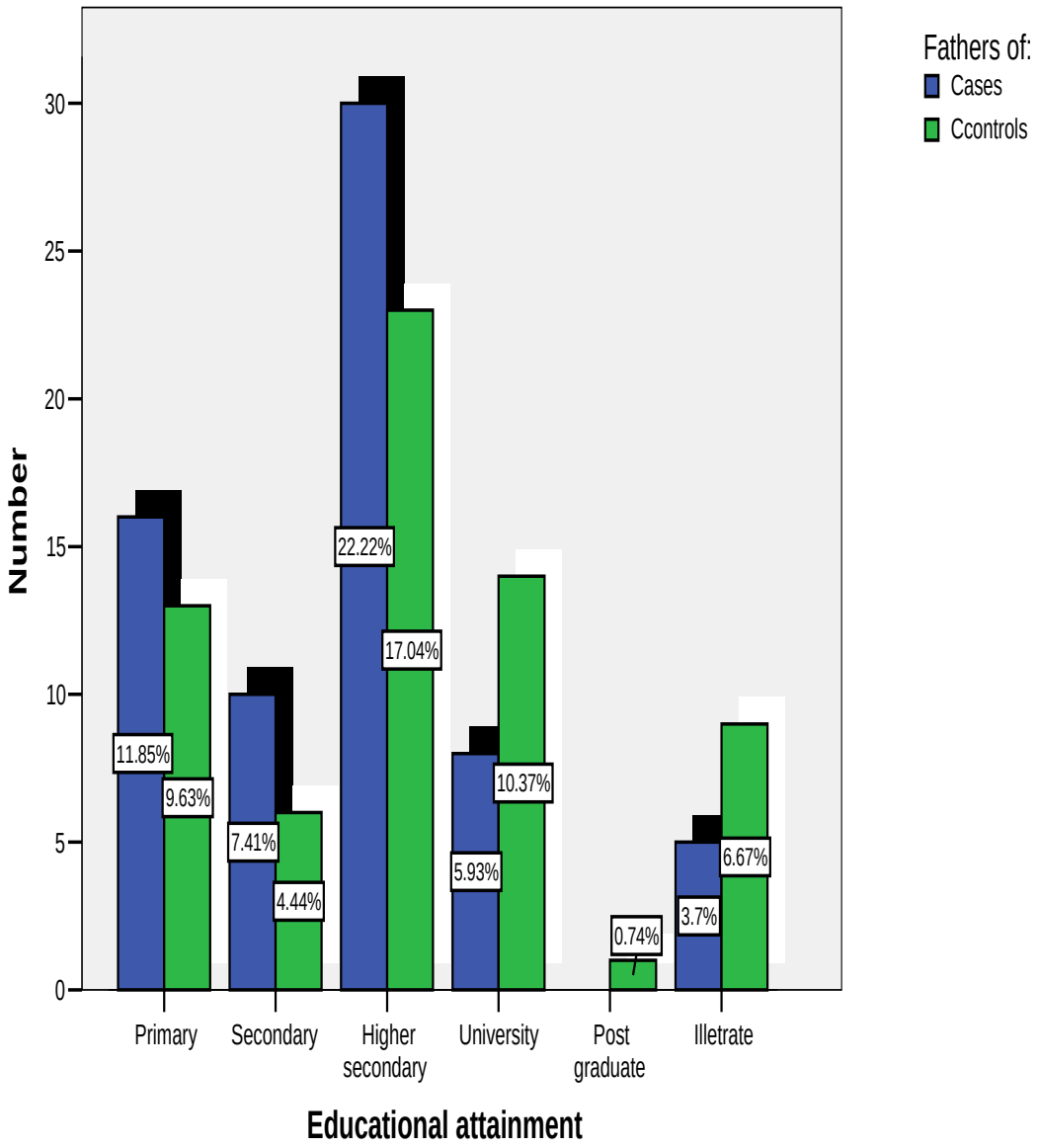


Fig 8: Educational Attainment of Mothers in the Study Group

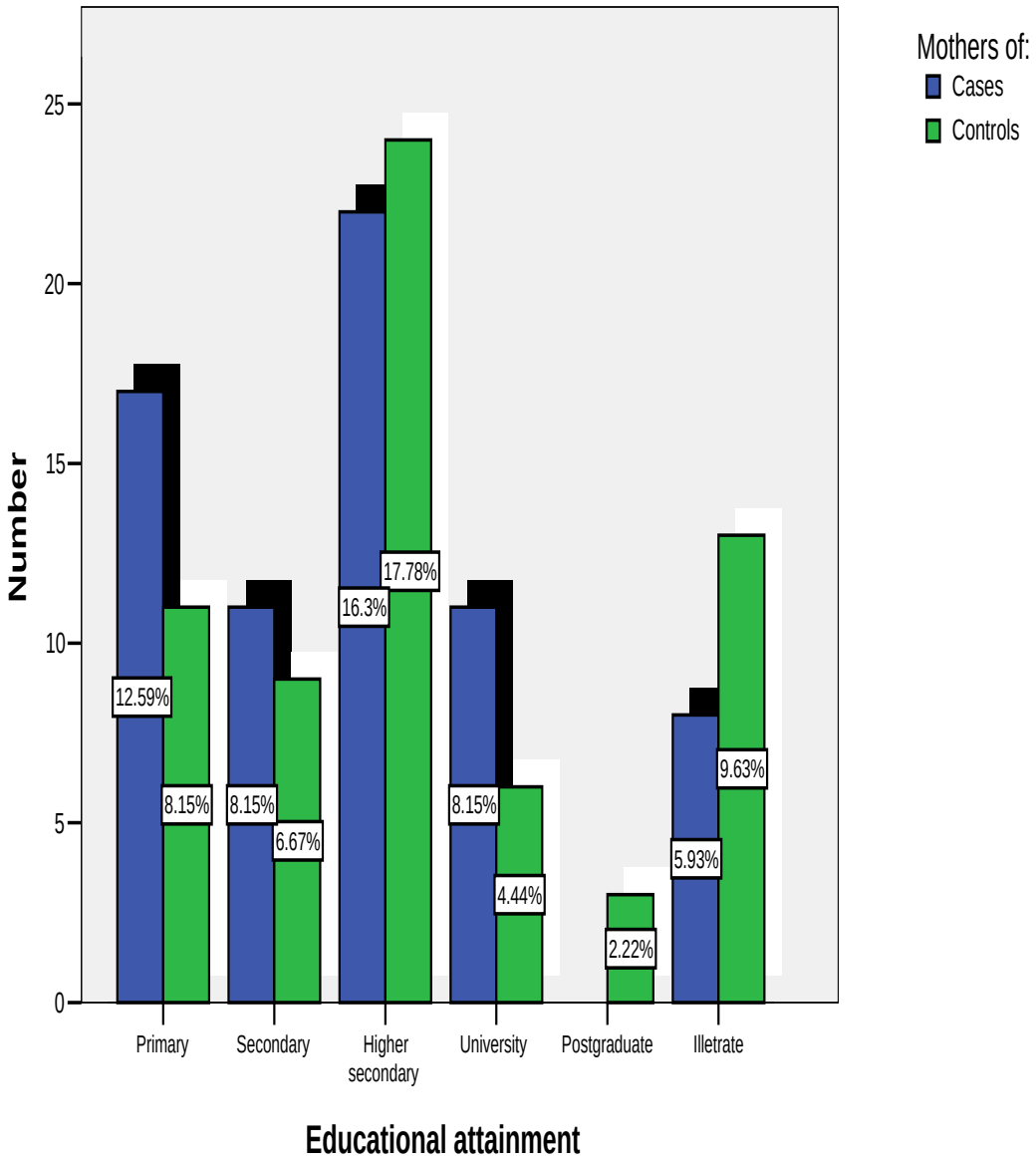


Figure 9: Knowledge of Fathers about Adverse Effects of Passive Smoking on Child's Health

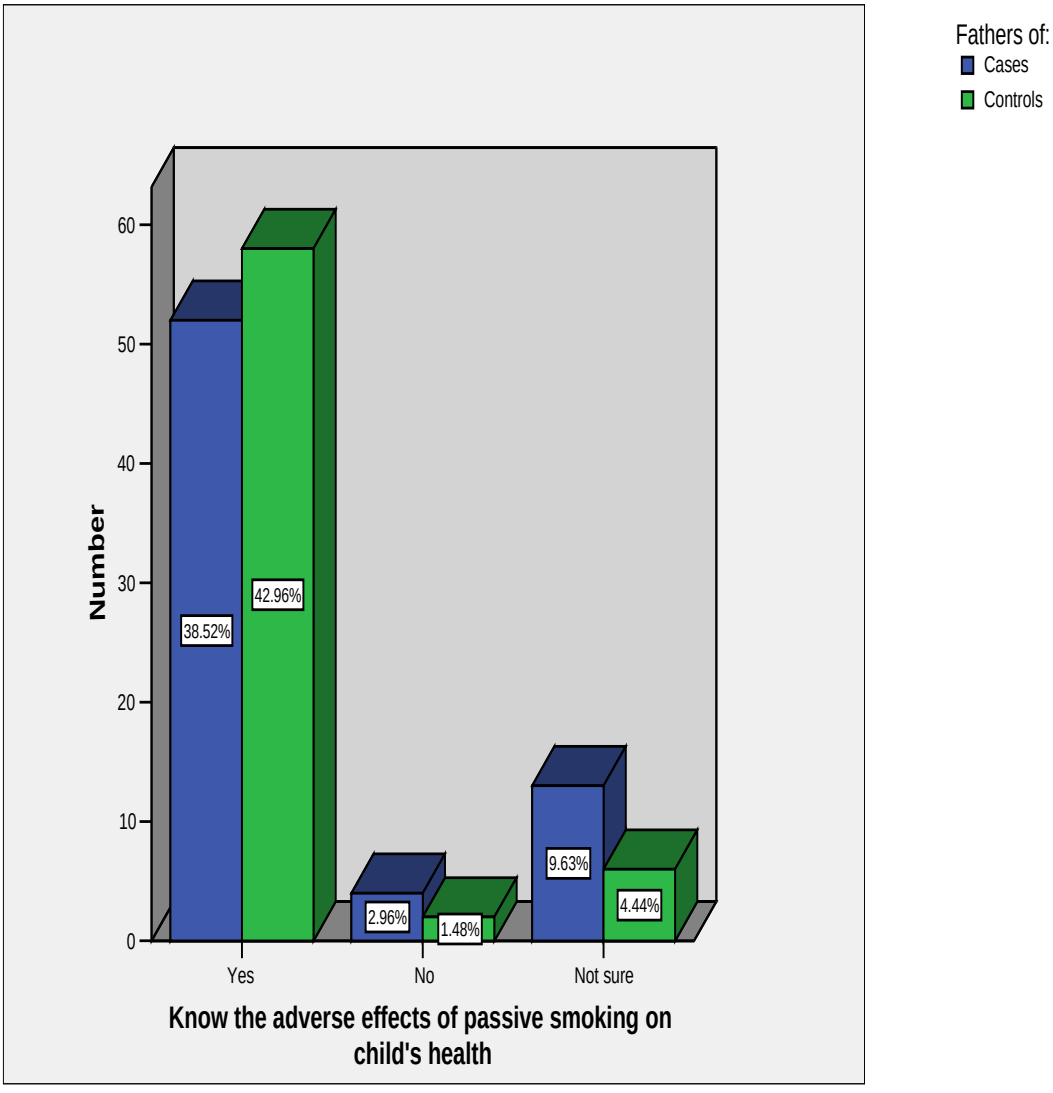
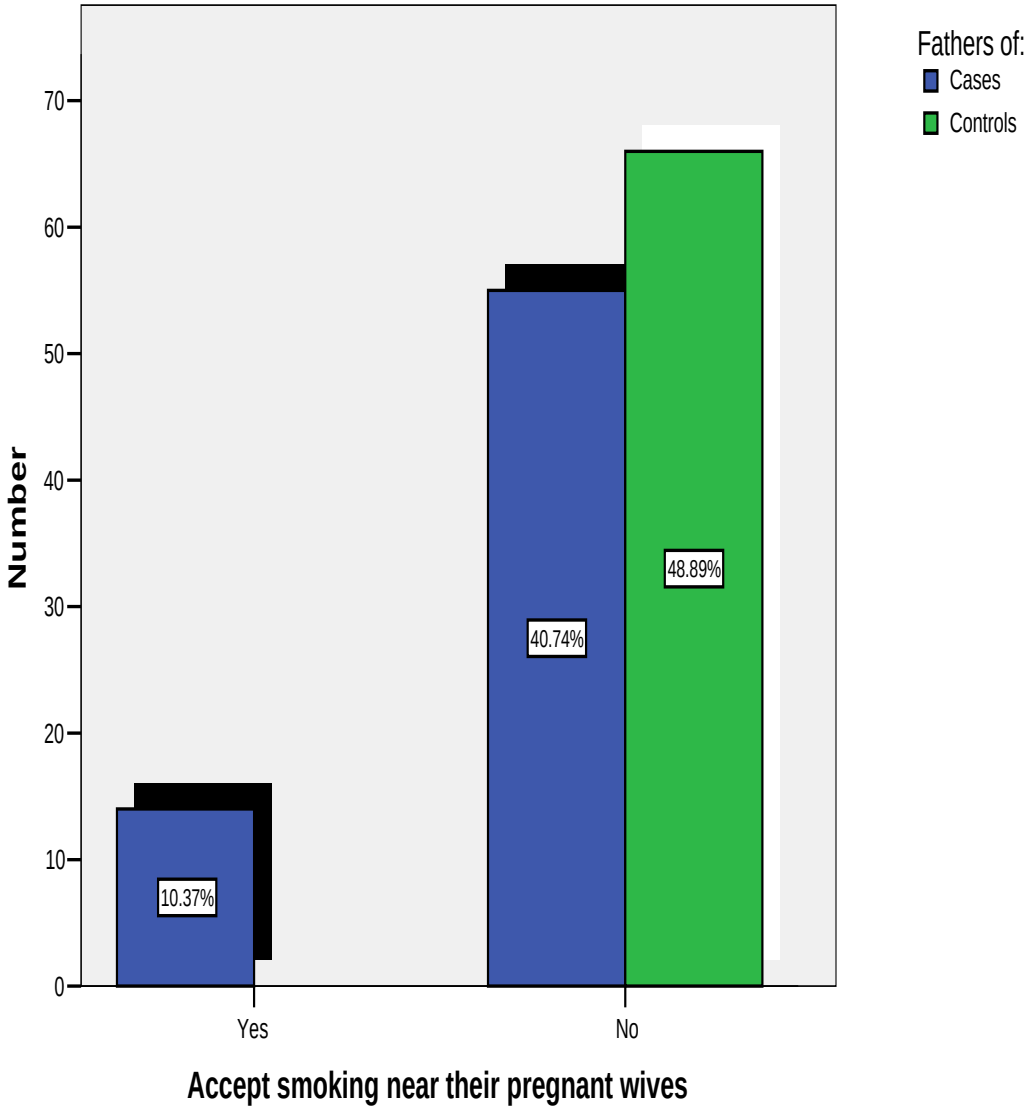


Figure 10: Attitude of Fathers towards Smoking near their Pregnant Wives



$P < 0.05$

Figure 11: Past History of Hospital Admissions among Cases and Controls in the Study Group

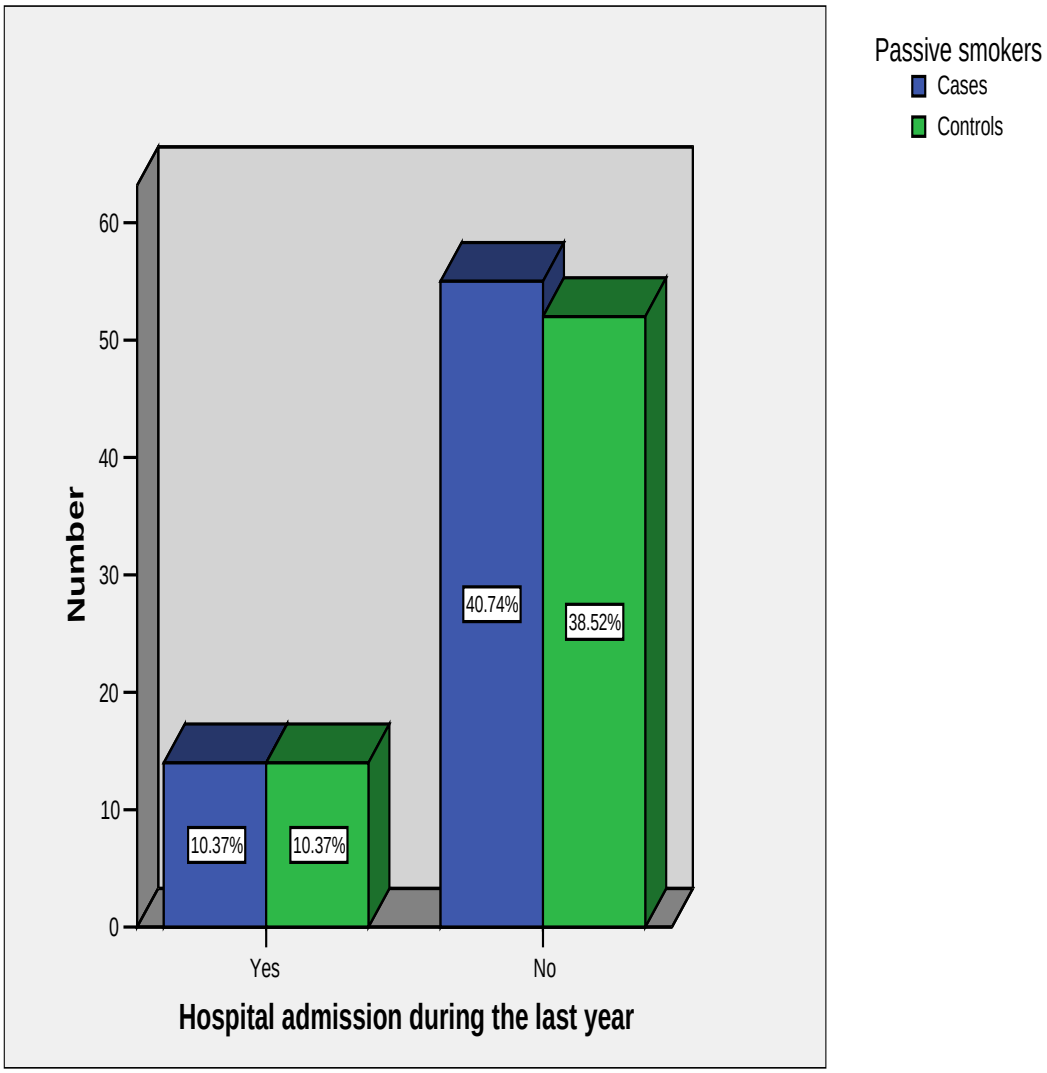


Figure 12: Past History of Surgical Operations among Cases and Controls in the Study Group

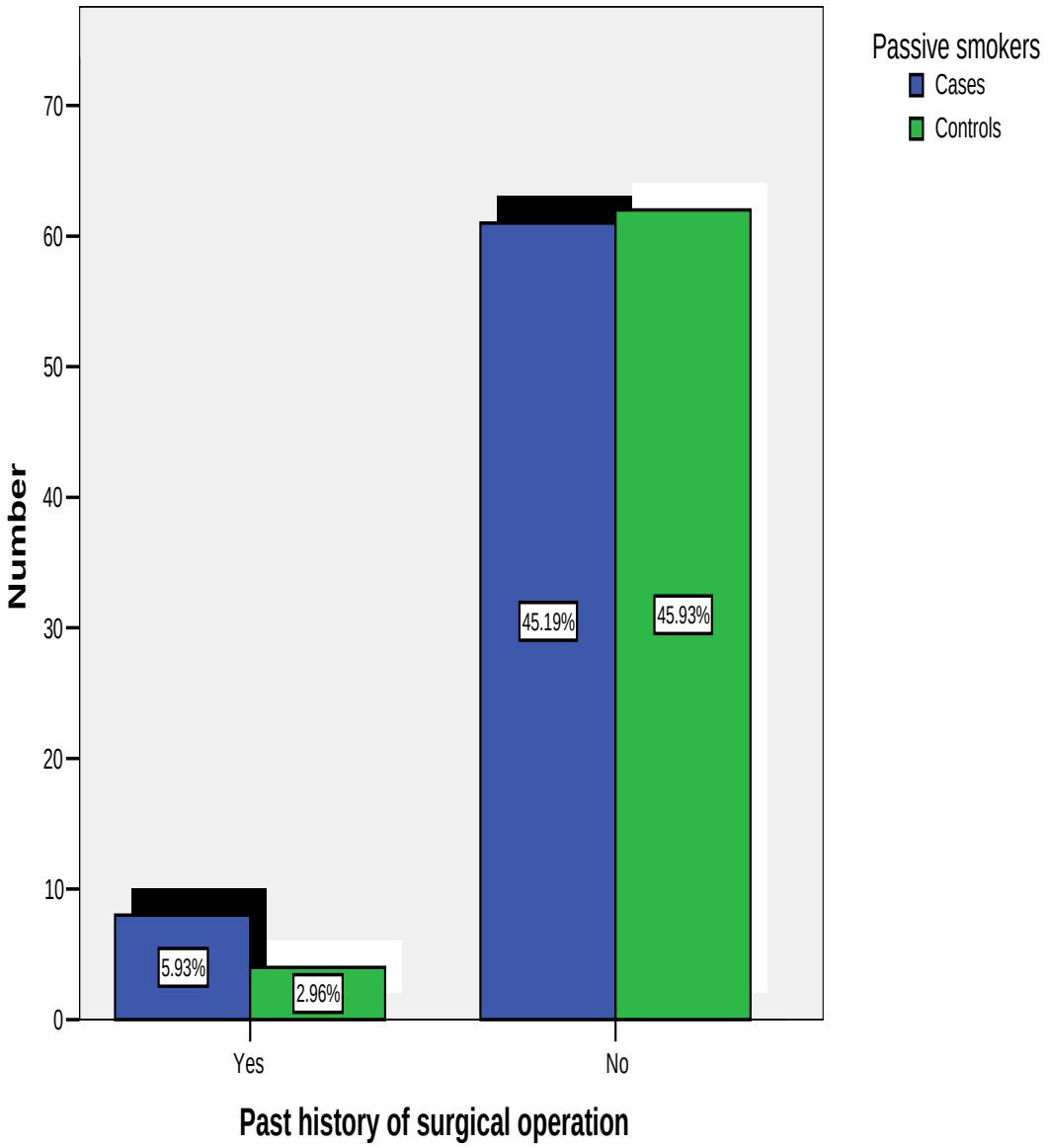


Figure 13: Illnesses during the Last Year among Cases & Controls in the Study Group

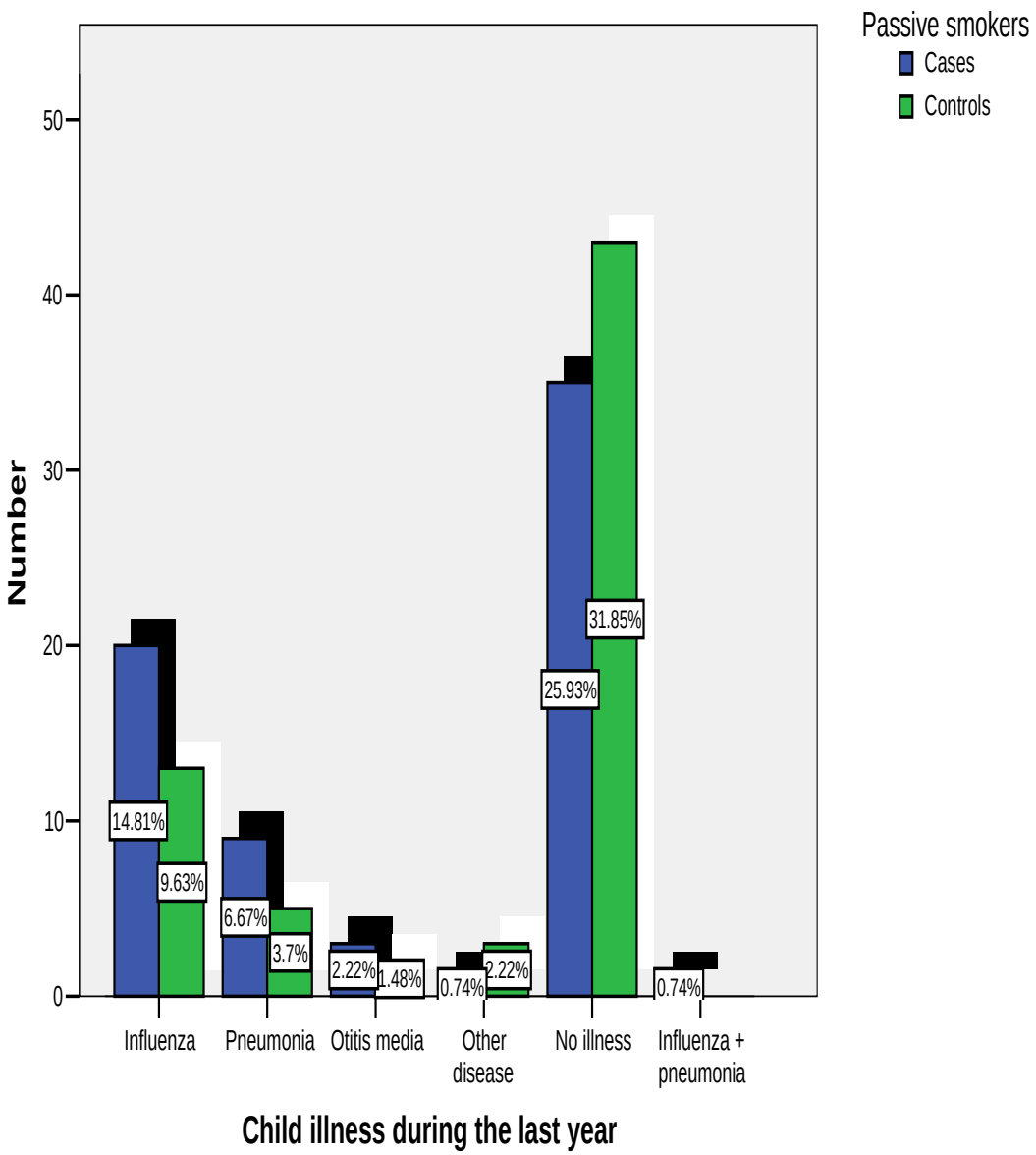
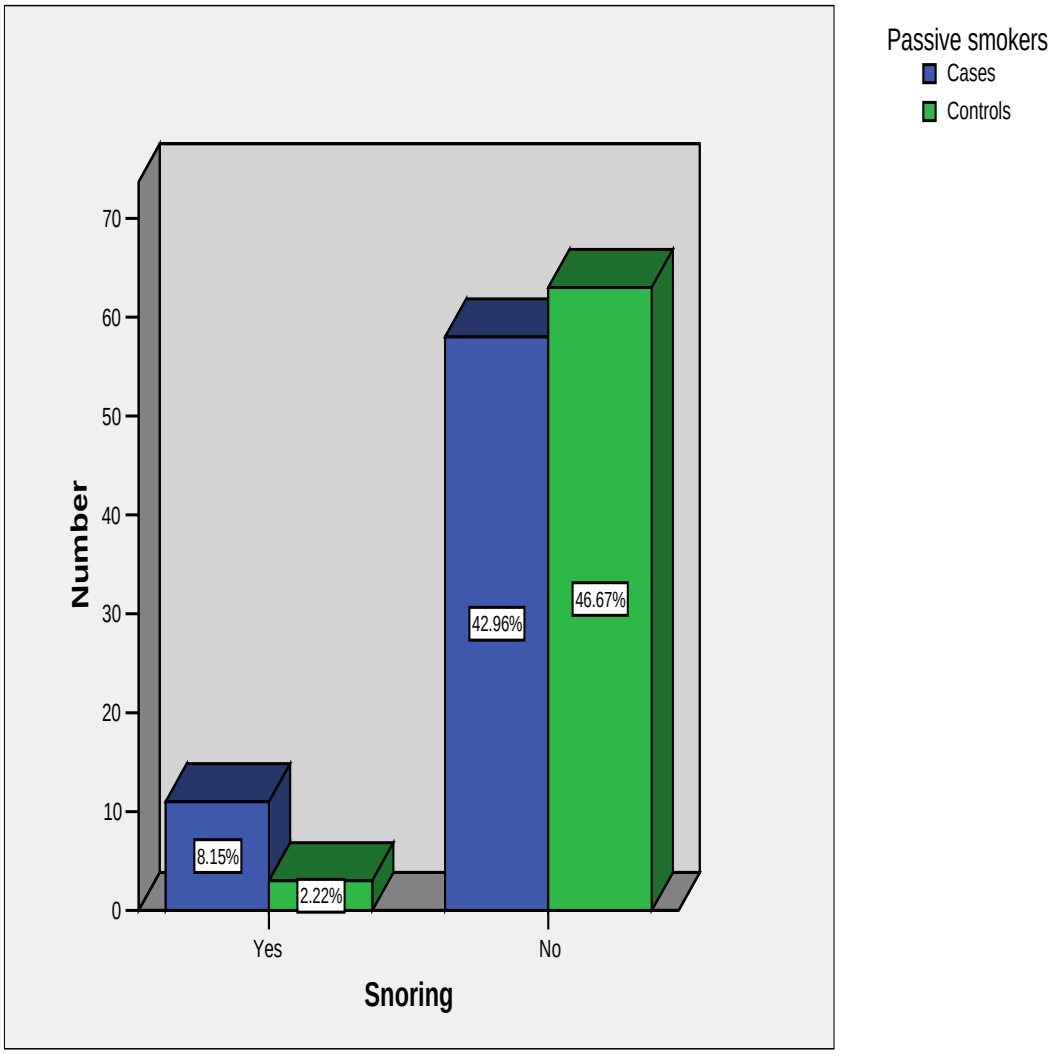
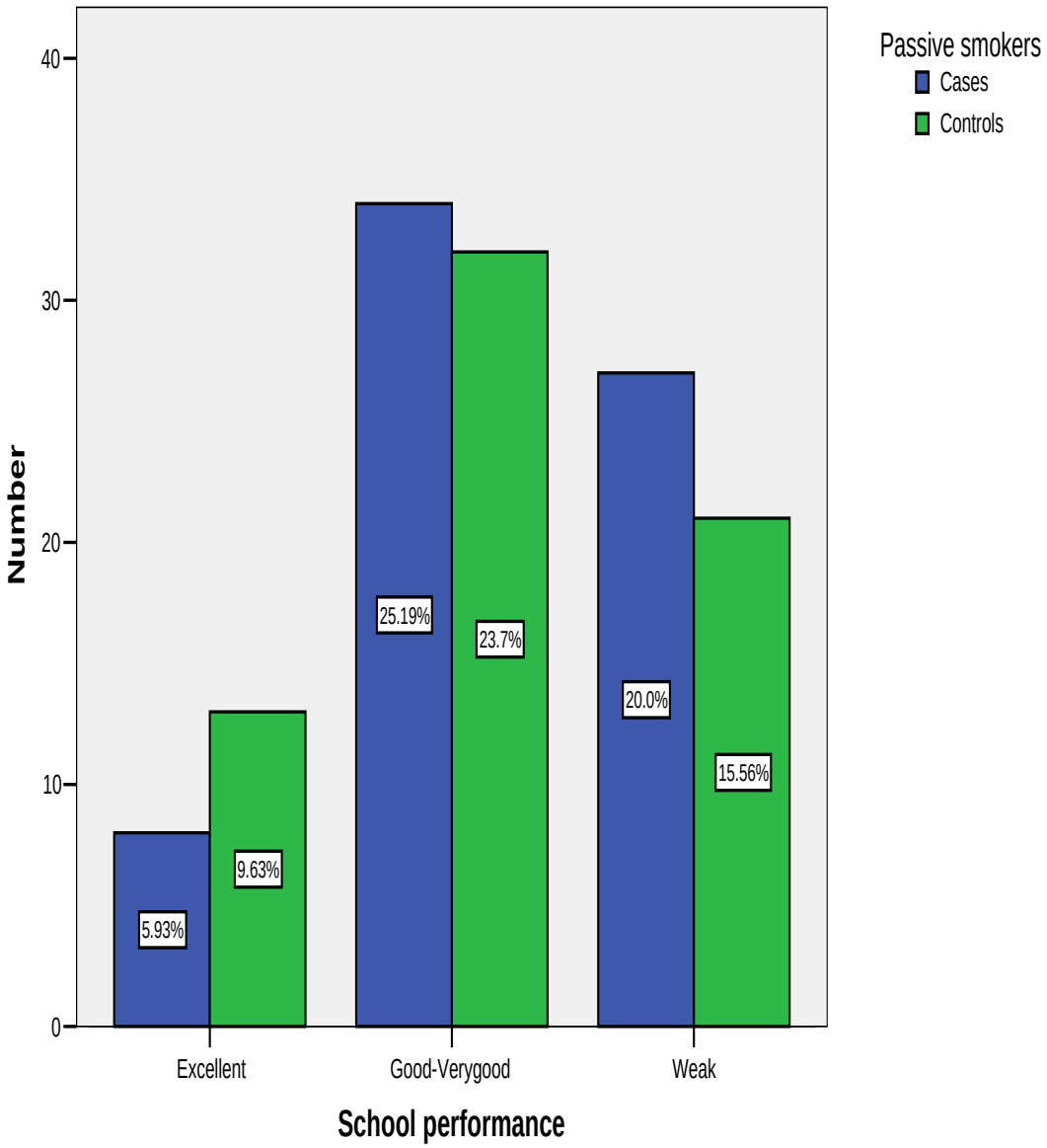


Figure 14: Sleep Snoring among Cases and Controls in the Study Group



$P < 0.05$

Figure 15: School Performance among Cases and Controls in the Study Group



CHAPTER FOUR

4. DISCUSSION

4.1 Effect of passive smoking on spirometric values

The effects of passive smoking on ventilatory functions of the lung in children and adolescents were assessed in many studies worldwide. The results of these studies were controversial. Some studies found significant association between passive smoking and impaired lung function (188-190) whereas others did not.(191-193) Some studies reported sex differences in vulnerability to the negative effects of passive smoking, with females being affected more than males; others reported different results and related the negative effects to maternal smoking rather than paternal smoking.(99-106) The variation in the effect of passive smoking on lung function in children at the school age were attributed to many reasons. It may be related to differences in genetic factors or variation in environmental factors, life styles and housing conditions of different communities.

In this study, the mean spirometric values (FVC, FEV_1, FEF_{50} & PEF) were all lower in children exposed to environmental tobacco smoke at home than those who were not exposed. The significant reduction in FVC and FEV_1 (by 7-8%) suggested that indoor environmental tobacco smoke did have negative effects on lung function of our male children. Although the results were lower in children with high degree of exposure than those with no or with mild-moderate degree of exposure, this effect was not statistically significant; however, it indicated a dose related effect of passive smoking on the degree of reduction in lung function. Genetic vulnerability of the children and inadequate ventilation of their housing environment could be involved in this reduction in lung function. The vulnerability of the lungs in Sudanese subjects to the adverse effects of respirable particles such as

cotton dust, flour dust and other particles was confirmed in many previous studies.(194-198) On the other hand, inadequate ventilation prevents exchange of the indoor air, which is saturated with tobacco smoke, with the outdoor air. This exchange is supposed to decrease the concentration of gases and particulates in tobacco smoke such as nicotine, polyacrylic hydrocarbons, carbon monoxide, acrolein and nitrogen dioxide; thus lowering the negative effects of passive smoking on lung function, but this needs further study to be confirmed.

4.2 Effect on plasma TNF-α

Cigarette smoke can impair pulmonary immune function and initiate release of inflammatory mediators and hence influences the development of lung diseases. Recent studies have shown that TNFα plays an important role in the induction of the cigarette related COPD and in the maintenance of airway inflammation.(199) Experimental studies in rats exposed to tobacco smoke confirmed the rise in TNFα in both serum and broncho-alveolar lavage fluid.(135,165)

In this study, a significant rise in plasma level of TNFα was detected in the group of passive smokers compared to the control group. This finding indicated that inflammation within the airways of passive smokers was established and there would be a risk of progression to COPD with continuous exposure to tobacco smoke. The contribution of passive smoking to the pathogenesis of COPD and its related mortality was not studied in Sudan; however, worldwide, passive smoking is considered as an important risk factor for 10-15% of COPD deaths that are not attributed to active smoking.(200, 201) In addition, a large proportion of the active

smokers who suffer from COPD have been passive smokers during their childhood.

4.3 Effect on plasma IL4

It was proposed that tobacco smoke modifies CD4/CD8 ratio of T cells, increases T helper cells type 2 and increases IL4/IFNγ ratio, either by increasing IL4 or decreasing IFNγ, resulting in activation of B cells to produce IgE antibodies.(151,152) El-nawawy and colleagues in 1996 conducted a research in Egypt on the effects of passive smoking on frequency of respiratory illnesses, serum IgE and IL4.(202) They found higher frequency of respiratory illnesses per year, significantly higher total leucocyte count, higher percentage of eosinophils, and higher serum IgE and IL4 concentrations in children of smoking parents compared to the control group. Oryszczynin et al. reported similar rise in IgE antibodies in adults.(203) In this study, the rise in plasma level of IL4 in passive smokers was statistically significant. On the other hand, a significant rise in plasma IL4 was detected in the pupils with high degree of exposure to tobacco smoke compared to the other pupils; again indicating a dose related effect.

4.4 Effect on plasma CRP

The acute phase protein "C-Reactive Protein (CRP)" is a well studied marker of systemic inflammation (145) and recently has been regarded as a predictor of cardiovascular disease.(130,149) Many studies reported higher level of CRP among people exposed to passive smoking,(146-148) leading to accelerated atherosclerosis and subsequently increased mortality rates in the middle and later years of life. In this study, although the mean plasma level of

CRP was slightly higher in the group of cases than the group of controls, the difference was statistically insignificant. However, since the plasma concentrations of the other cytokines TNFα and IL4 were found to be significantly elevated, the insignificant rise in plasma CRP did not exclude presence of inflammatory process within the airways.

4.5 Knowledge and attitude of parents towards passive smoking

In this study, it was confirmed that the adverse health effects of passive smoking on children and adolescents were known to most parents in the study group, irrespective of their educational attainment. However, a significant difference was detected in the attitude of fathers of the two groups (cases and controls) towards smoking near their pregnant wives. This finding suggested that the knowledge about the negative effects of passive smoking on child's health did not prevent fathers in the group of cases from smoking near their children or near their wives during pregnancy. Similar results were reported by Liem and his colleagues (204) who found that parent's of asthmatic children do not quit smoking or stop smoking at home in the presence of an asthmatic child. Moreover, many randomized controlled trials with interventions to ban parental smoking at home found that parents are unlikely to change their behavior.(205-208)

4.6 Effect of passive smoking on child's health

Children exposed to environmental tobacco smoke at home, compared to those who are not exposed, have higher frequency of hospital admissions due to repeated illnesses (63) and higher incidence of influenza, pneumonia and bronchitis.(64-65) In addition, middle ear problems that need surgical

treatment are more likely to occur in children who live in households where more than three packs of cigarettes are smoked per day.(70)

In this study, no significant difference in the frequency of hospital admission between children in the group of cases and those in the group of controls was detected. In addition, no significant difference was found in the frequency of upper respiratory tract infection, pneumonia, otitis media or surgical interventions between the two groups. Further cross sectional studies with larger sample sizes may be needed to test the effect of passive smoking on child's health.

4.7 Effect of passive smoking on sleep

Habitual snoring during sleep is a well known complication of smoking in both children and adults. Many studies described smoking-snoring association in passive smokers as well as active smokers.(209-212) The association was found to be independent of both obesity and sex.(213) In this study, a significant relation was found between passive smoking and snoring during sleep. The percentage of pupils who were suffering from snoring was significantly higher in the group of cases than in the group of controls. However, the exact mechanism by which tobacco smoke induces snoring is unknown. Smoking-induced inflammatory damage to mucosal neural mechanisms that are supposed to act against snoring is a possibility.(213) However, the current medical research is expected to clarify this area in the future.

4.8 Effect of passive smoking on academic performance

The negative effects of passive smoking on cognitive function and mental development are well known late complications in adults. Passive smoking has also been associated with learning difficulties, behavior problems, and language difficulties in childhood;(214) however, further work is needed to clarify the exact mechanism of this association. In this study, the percentage of pupils with excellent academic performance in the last school examination was higher among controls than cases whereas the percentage of those with weak academic performance was higher among cases than controls. These findings were not different from findings in many other studies worldwide. For example, Yolton et al. found deficits in reading and reasoning skills among children even at low levels of tobacco smoke exposure.(85)

In this study, effects of passive smoking on spirometric values and plasma level of inflammatory markers were tested among healthy pupils of "governmental schools" for boys; however, the effects on girls are not studied. On the other hand, the effects of passive smoking on pupils of "private schools" need to be documented because the difference in socioeconomic status of pupils in the two types of school may influence the mean values of the results. Previous studies showed that high socioeconomic factors operating from early life, including the overall household income, affect the adult FEV_1 and the risk of developing COPD independently of smoking in both males and females.(215) On the other hand, low socioeconomic status has recently been related to higher levels of inflammatory markers.(216,217) Further research is needed to quantify

the influence of socioeconomic factors on lung function and inflammatory markers in both males and females.

CONCLUSION

1- Passive smoking can initiate inflammatory reaction and elevate the plasma level of the inflammatory markers IL4 and TNF. The response is accelerated when the dose of passive smoking is high.

2- Passive smoking has a significant negative impact on lung function of healthy school pupils in Khartoum. It causes 7-8% reduction in the spirometric parameters FVC and FEV_1.

3- Most parents of school pupils in Khartoum have knowledge about the adverse effects of passive smoking on child's health.

4- Knowledge about the negative effects of passive smoking on child's health does not prevent fathers from smoking near their children or near their wives during pregnancy.

5- Passive smoking is not associated with higher frequency of hospital admission among school pupils in Khartoum.

6- Passive smoking is not associated with higher incidence of upper respiratory tract infection, pneumonia, otitis media or surgical interventions among school pupils in Khartoum.

7- Passive smoking in school-pupils has a significant relation to snoring during sleep.

8- Passive smoking in school pupils is associated with weaker academic performance.

RECOMMENDATIONS

1- Educational programs are highly recommended to increase awareness of parents about the negative impact of passive smoking on child's health in order to decrease second-hand smoke indoors.

2- Public health policies are needed to protect children from cigarette smoke of their parents.

3- Further scientific research should be promoted to investigate other risks associated with passive smoking.

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APPENDIX

بسم الله الرحمن الرحيم

DEPARTMENT OF PHYSIOLOGY
FACULTY OF MEDICINE
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كلية الطب
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ص.ب. 102
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بحث في وظائف الرئة لدى التلاميذ

استبيان رقم (1) – أسئلة للتلميذ

الرقم: ( ) _____ **المجموعة:** ( )

1. عمر التلميذ: .....
2. المرحلة الدراسية: .....
3. آخر تقدير أكاديمي في الصف: .....
4. هل تدخن السجائر:

   نعم يومياً ( ) نعم احياناً ( ) لا ( )
5. هل هناك من يدخن السجائر من المقيمين بالمنزل

   نعم ( ) لا ( )

إذا كانت الإجابة بنعم حدد .....

الوالد ( ) الوالدة ( )

احد الاخوة ( ) احد الاقارب ( ) حدد .....

6. هل تشم أو تتعرض لرائحة السجائر بالمنزل

   نعم يومياً ( ) نعم أحياناً ( ) لا ( )

إذا كانت الإجابة بنعم حدد عدد السجائر في اليوم ( ) وعدد السنوات ( )

7. هل تشم أو تتعرض لرائحة السجائر خارج المنزل؟ .....

   نعم يومياً ( ) نعم احياناً ( ) لا ( )

إذا كانت الإجابة بنعم حدد المصدر .....

8. هل تشم أو تتعرض لرائحة أي دخان آخر داخل أو خارج المنزل؟

   نعم ( ) لا ( )

إذا كانت الإجابة بنعم حدد المصدر .....

9. هل تعاني من أي مرض حالياً؟

نعم ◯ لا ◯

حدد .....

10. هل تعاني من ازمة او صفير بالصدر خلال الاثني شهرا السابقة؟

نعم ◯ لا ◯

---

11. الكشف السريري:

طبيعـ◯ـي ◯ـ ـ ـ ـر طبيعـ ـ ـ ـ ـي حـ ـ ـ ـ ـدد

.....

12. الطول

13. الوزن

14. BMI

---

15. وظائف الرئة (a):

FVC	FEV1	(%)	PEF	FEF 50	
1-.....	.....	.....	.....	.....	.....
2-	.....	.....	.....	.....	.....
3-	.....	.....	.....	.....	.....
4-	.....	.....	.....	.....	.....
5-	.....	.....	.....	.....	.....
6-	.....	.....	.....	.....	.....

16. CRP

17. IL4

18. TNF

19. اختبارات اخرى:

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بحث في وظائف الرئة لدى التلاميذ
أستبيان رقم (2) – أسئلة للوالد

الرقم: ( )

1. العمر: .....
2. الموطن الأصلي: .....
3. القبيلة: .....
4. المرحلة التعليمية: .....
5. الوظيفة: .....
6. هل تكلفة علاج اسرتك مغطاة بالتأمين الصحي؟ نعم ◯ لا ◯
7. إذا كانت الإجابة لا فهل دخل الاسرة يكفي بصورة عامة لتغطية تكلفة العلاج
   نعم ◯ لا ◯
8. هل تدخن السجائر؟ .....

   نعم وما زلت أدخن ◯ نعم ولكن في الماضي ◯ لا أبداً ◯
9. اذا كانت الاجابة (نعم أو نعم ولكن في الماضي) فاجب علي الاتي:
   - o كم المدة التي دخنت فيها بالسنوات ( )
   - o كم عدد السجائر المدخن في اليوم ( )
   - o اذكر نوع السجائر المستخدم ( )
   - o متي توقفت عن التدخين بالسنوات ( )

10. هل كنت او ما زلت تدخن في البيت؟: نعم ◯ أبدا ◯

11. اذا كانت الاجابة نعم فكم عدد السجائر المدخن في البيت في اليوم ◯

12. هل تدخن في وجود الابناء؟

نعم باستمرار ◯ نعم احياناً ◯ لا أبداً ◯

13. هل تؤمن بوجود مخاطر على الطفل عند التدخين أمامه؟

نعم ◯ ◯ لا ◯ لا أعلم

14. هل دخنت السجائر أمام والدة التلميذ أثناء فترة الحمل به؟

نعم ◯ لا ◯

أستبيان رقم (3) – أسئلة للوالدة

15. العمر: .....

16. المرحلة التعليمية: .....

17. الوظيفة: .....

18. هل تدخنين السجائر؟

نعم وما زلت أدخن ◯ نعم ولكن في الماضي ◯ لا أبداً ◯

- اذا كانت الاجابة لا فانتقلي مباشرة الي السؤال رقم 26
- اذا كانت الاجابة (نعم أو نعم ولكن في الماضي) فاجبي علي الاتي:

19. كم المدة التي دخنت فيها بالسنوات ◯

20. كم عدد السجائر المدخن في اليوم ◯

21. اذكري نوع السجائر المستخدم ◯

22. متي توقفت عن التدخين بالسنوات ◯

23. هل كنتي او ما زلتي تدخنين في البيت؟:

نعم ◯ لا أبدا ◯

24. اذا كانت الاجابة نعم فكم عدد السجائر المدخن في البيت في اليوم ◯

25. هل تدخنين في وجود الطفل؟

نعم باستمرار ◯ نعم احياناً ◯ لا أبداً ◯

26. هل تؤمنين بوجود مخاطر على الطفل عند التدخين أمامه؟

نعم ◯ لا ◯ لا أعلم ◯

27. هل حدث ان استنشقت السجائر أثناء فترة الحمل بالتلميذ؟

نعم ◯ لا ◯ لست متأكدة ◯

28. كم وزن التلميذ عند ولادته؟

2.5 كجم أو أكثر ◯ أقل من 2.5 ◯ لا أتذكر ◯

أستبيان رقم (4) – أسئلة للوالدين عن صحة التلميذ

29. هل التلميذ يعاني من اي مرض حاليا؟ ◯نعم ◯ لا

اذا كانت الاجابة نعم فما هو المرض؟ .....

30. هل ادخل التلميذ المستشفي لأكثر من 24 ساعة خلال حياته لأي سبب؟

نعم ◯ لا ◯ اذا نعم فما هو السبب .....

31. هل اجريت للتلميذ أي عملية جراحية في السابق؟

نعم ◯ لا ◯ اذا نعم فما هو السبب .....

32. هل أصيب التلميذ بأي من الأمراض الاتية خلال الثلاث سنوات الماضية:

- انفلونزا نعم ◯ لا ◯ اذا نعم، كم مرة في الس
- التهاب رئوي نعم ◯ لا ◯ اذا نعم، كم مرة في الس
- التهاب بالاذن الوسطي نعم ◯ لا ◯ اذا نعم، كم مرة في السن
- أي مرض اخر نعم ◯ لا ◯ ما

33. هل التلميذ يعاني من نوبات أزمة أو صفير بالصدر خلال الأثني عشر شهرا السابقة؟

نعم ◯ لا ◯

34. هل التلميذ يعاني من حساسية بالصدر أو أي مكان اخر بالجسم:

نعم ◯ لا ◯ حدد .....

35. هل بالاسرة حالات أزمة؟

نعم ◯ لا ◯

36. هل بالاسرة حالات حساسية بالانف أو الجلد؟

نعم ◯ لا ◯

- *نرجو موافقتكم علي اجراء اختبار وظائف الرئة لابنكم وأخذ عينة دم للفحص*

- *سيقوم بالاختبار اختصاصي في أمراض الصدر ووظائف الاعضاء من كلية الطب* جامعة الخرطوم

- لا نوافق علي اجراء الاختبارات ⬭
- نوافق علي اجراء الاختبارات ⬭ امضاء ولي الامر .....
- عنوان المنزل: .....
- تلفونات: .....